Universidade de Aveiro Departamento de Química
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Anabela Ferreira de Oliveira Cachada

Contaminantes orgânicos em solos urbanos: fontes e potenciais riscos

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Tese apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Doutor em Química, realizada sob a orientação científica do Doutor Armando da Costa Duarte, Professor Catedrático do Departamento de Química da Universidade de Aveiro e do Doutor Eduardo Anselmo Ferreira da Silva, Professor Catedrático do Departamento de Geociências da Universidade de Aveiro.

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Dedico este trabalho aos meus avós

o júri

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palavras-chave

Análise geostatística; análise de risco; biodisponibilidade, disponibilidade química, estatística multivariada; PAHs, PCBs, saúde ambiental; saúde humana; solos urbanos; identificação de fontes; poeiras.

resumo

A qualidade dos solos urbanos pode ser afetada por contaminantes orgânicos hidrofóbicos (HOCs), prejudicando a saúde ambiental e humana. Este trabalho consistiu em estudar duas áreas urbanas contrastantes (Lisboa e Viseu), com o objetivo de avaliar os níveis de HOCs nos solos e os seus potenciais riscos para a saúde humana e para o ambiente. Pretendia-se ainda identificar as fontes e estudar o comportamento destes contaminantes no solo. Foi possível relacionar as concentrações de HOCs com o tamanho da cidade, sendo os níveis de contaminação muito mais elevados em Lisboa. A identificação das fontes destes contaminantes foi feita através do estudo dos respetivos perfis e da relação com elementos potencialmente tóxicos, utilizando métodos estatísticos multivariados. Lisboa parece ser afetada por fontes próximas (tráfego, indústria e incineração) enquanto em Viseu o transporte atmosférico aparenta ter um papel mais importante. Num primeiro nível da avaliação de risco (RA), foi possível identificar os hidrocarbonetos aromáticos policíclicos (PAHs) nos solos de Lisboa como um perigo potencial. Os níveis de PAHs em poeiras das ruas de Lisboa foram também estudados e permitiram clarificar que o tráfego e os detritos de pneus e de pavimento podem também ser uma importante fonte destes compostos. Utilizaram-se e discutiram-se ferramentas de geoestatística assim como a respetiva utilidade em RA e em planeamento urbano. De modo a obter uma avaliação mais realista dos riscos de HOCs é importante avaliar a fração disponível, que é também a mais acessível para os organismos. Deste modo, foi feita uma avaliação dos processos envolvidos na disponibilidade de PAHs e também dos resultados obtidos pelos diferentes métodos químicos. A adequação dos métodos químicos para prever a biodisponibilidade de PAHs em solos naturalmente contaminados ainda não foi demonstrada, sendo especialmente difícil para os compostos de elevado peso molecular. No presente trabalho também não foi possível estabelecer uma relação significativa entre a disponibilidade química e a biodisponibilidade. No entanto, apesar das elevadas concentrações totais encontradas em alguns solos de Lisboa, tanto a fração solúvel em água como os resíduos acumulados nos ensaios de bioacumulação foram, em geral, muito baixos, o que estará relacionado com os fenómenos de envelhecimento destes contaminantes nos solos. Observou-se que a fração solúvel de PAHs depende do composto em causa e é regulada pelas propriedades do solo. Apesar de não se terem observado correlações entre as propriedades do solo e a biodisponibilidade, observou-se que os fatores de bioacumulação dependem mais da amostra do que do composto. Em conclusão: após a identificação dos contaminantes de interesse uma avaliação química baseada nos teores totais pode ser uma abordagem eficaz no primeiro nível da RA, mas no entanto é necessário melhorar os modelos existentes para a caracterização do risco.

keywords

Bioavailability, chemical availability, environmetal health; geostatistical analysis; multivariate statistics; PAHs, PCBs, urban soils; human health; risk assessment; source apportioning; street dust.

abstract

Urban soil quality may be severely affected by hydrophobic organic contaminants (HOCs), impairing environmental quality and human health. A comprehensive study was conducted in two contrasting Portuguese urban areas (Lisbon and Viseu) in order to assess the levels and potential risks of these contaminants, to identify sources and study their behaviour in soils. The concentrations of HOCs were related to the size of the city, with much higher contamination levels observed in Lisbon urban area. Source apportionment was performed by studying the HOCs profiles, their relationship with potentially toxic elements and general characteristics of soil using multivariate statistical methods. Lisbon seems to be affected by nearby sources (traffic, industry and incineration processes) whereas in Viseu the atmospheric transport may be playing an important role. In a first tier of risk assessment (RA) it was possible to identify polycyclic aromatic hydrocarbons (PAHs) in Lisbon soils as a potential hazard. The levels of PAHs in street dusts were further studied and allowed to clarify that traffic, tire and pavement debris can be an important source of PAHs to urban soils. Street dusts were also identified as being a potential concern regarding human and environmental health, especially if reaching the nearby aquatic bodies. Geostatistical tools were also used and their usefulness in a RA analysis and urban planning was discussed.

In order to obtain a more realistic assessment of risks of HOCs to environment and human health it is important to evaluate their available fraction, which is also the most accessible for organisms. Therefore, a review of the processes involved on the availability of PAHs was performed and the outputs produced by the different chemical methods were evaluated. The suitability of chemical methods to predict bioavailability of PAHs in dissimilar naturally contaminated soils has not been demonstrated, being especially difficult for high molecular weight compounds. No clear relationship between chemical and biological availability was found in this work. Yet, in spite of the very high total concentrations found in some Lisbon soils, both the water soluble fraction and the body residues resulting from bioaccumulation assays were generally very low, which may be due to aging phenomena. It was observed that the percentage of soluble fraction of PAHs in soils was found to be different among compounds and mostly regulated by soil properties. Regarding bioaccumulation assays, although no significant relationship was found between soil properties and bioavailability, it was verified that biota-to-soil bioaccumulation factors were sample dependent rather than compound dependent. In conclusion, once the compounds of potential concern are targeted, then performing a chemical screening as a first tier can be a simple and effective approach to start a RA. However, reliable data is still required to improve the existing models for risk characterization.

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ABBREVIATIONS

A

ACE , acenaphthene

ACY, acenaphthylene

AF, adherence factor soil to skin

ANT, anthracene

AT, averaging time

B

BAA, benzo(a)anthracene

BAP, benzo(a)pyrene

BAPeq, benzo(a)pyrene equivalents

BBF, benzo(b)fluoranthene

BC, black carbon;

BCF, bioconcentration factor

bdl, below detection limit

BGHI, benzo(ghi)perylene

BKF, benzo(k)fluoranthene

BSAF, biota-to-soil accumulation factor

BuOH, n-butanol

Bw, body weight

C

CA, cluster analysis

CAM, concentration addition model

CBR, critical body residue

CCME, Canadian Council of Ministers of the Environment

CEC, cation exchange capacity

CEV, critical exposure value

C_{free}, dissolved concentration in pore water

C_{org}, concentrations in an organism

CPS, equilibrium concentration in the sampler

CRY, chrysene

CSF_O, chronic oral slope factor

C_{soil}, concentration in soil

D

DBAH, dibenzo(ah)anthracene

DCM, dichloromethane

DSFad, soil dermal contact factor adjusted

dw, dry weight

E

EC, environmental concentrations

EC_x, effect concentration

ED, exposure duration

EF, exposure frequency

OECD, Organisation for Economic Cooperation and Development

EqPT, equilibrium partition theory

ERA, environmental risk assessment

ET, exposure time

EtOH, ethanol

EU, European Union

Exp, exposure

F

FA, factor analysis

FLA, fluoranthene

FLU, fluorene

F_{rap}, rapid desorbing fraction

fw, fresh weight

G

GC-MS, gas chromatograph–mass spectrometer

GIABS, fraction of contaminant absorbed in gastrointestinal tract

H

HC, hazard concentration

HC-PCBS, high chlorinated polychlorobiphenyls

HHRA, human health risk assessment

HI, hazard Index

HQ, hazard quotient

HMW, high molecular weight

HOC, hydrophobic organic contaminant

HU, hazard units

I

IFSad, soil ingestion factor adjusted

IND, indeno(1,2,3-cd)pyrene

IR, intake rate

IRS, soil ingestion rate

IUR, inhalation unit risk

IV, intervention value

K

K_{disk}, partition coefficient between disks and soils

K_{OC}, organic carbon-water partition coefficient

K_{OW}, octanol-water partition coefficient

L

Lip, lipids

LMW, low molecular weight

LOEC, lowest observed effect concentration

LOD, limit of detection

LR, lifetime risks

M

MeOH, methanol

MGP, manufactured gas plant

MPC, maximum permissible concentration

N

NC, negligible concentration

NOEC, non observed effect concentration

NP, naphthalene

O

OC, organic carbon

OM, organic matter

P

PAH, polycyclic aromatic hydrocarbon

PCB, polychlorobiphenyl

PEF, particle emission factor

PHE, phenanthrene

PNEC, predicted no effect concentration

POM-SPE, polyoxymethylene solid phase extraction

PrOH, propanol

PTE, potentially toxic element

PYR, pyrene

R

RA, risk assessment

RAM, response addition model

RfC, inhalation reference concentrations

RfD_O, oral reference dose

RSD, relative standard deviation

S

SA, surface area

SFE, supercritical fluid extraction

SOM, soil organic matter

SPE, solid phase extraction

SPME, solid phase microextraction

SQG, soil quality guideline

SRC, serious risk concentration

SSD, species sensitivity distributions

STD, standard deviation

SWE, subcritical water extraction

T

TC, total carbon

TDI, tolerable daily intake

TECAMs, triolein embeddeded cellulose acetate membranes

TEF, toxic equivalent factor

TLCR, total lifetime cancer risk

TMoA, toxic mode of action

TR, target risk

TU, toxic unit

TUm, toxic units of the mixture

TV, target value

U

UCL, upper confidence level

USEPA, United States Environmental Agency

W

WHC, water holding capacity

Chapter 1

INTRODUCTION AND AIMS

1.1 BACKGROUND

Soil can be defined as "*the collection of natural bodies occupying parts of the Earth's surface that is capable of supporting plant growth and that has properties resulting from the integrated effects of climate and living organisms acting upon parent material, as conditioned by topography, over periods of time*" or in a simplest way "a *dynamic natural body composed of mineral and organic solids, gases, liquids, and living organisms*" (Brady and Weil, 2008). It performs several ecosystem services (key functions) such as food and biomass production; habitat for a variety of organisms; water filter and storage; nutrients cycling; carbon storage; and human's life support. In urban areas it has the additional function of supporting urban development. Moreover, it has impacts on environmental, economic and cultural activities. For all these reasons, soil is considered a vital resource, and due to its slow formation, it can be considered non-renewable. Thus, assessing soil quality is a very important issue but it can be challenging due to its natural spatial heterogeneity. Indeed, there are many definitions of soil quality (Bone et al., 2010), being one of the most used from Karlen et al. (1997): "*the capacity of a specific kind of soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation*". Therefore, soils quality is associated with soils' fitness for a specific use, and thus other authors suggest the term soil health which is "*the capacity of soil to function as a vital living system, within ecosystem and land-use boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and promote plant and animal health*" (Doran and Zeiss, 2000).

The ecological functions of soils may be strongly affected by different anthropogenic pressures and according to the EU Soil Thematic Strategy, some of the major threats for soil in Europe are compaction, point and diffuse contamination, and sealing (EC, 2006). These threats are more evident in urban areas, and due to the great urban growth, challenges regarding soil pollution have become very important. Particularly diffuse pollution (e.g. the redistribution of contaminants in soils and dust and long-range atmospheric transport), which is normally characterized by continuous and long-term emission of contaminants below risk levels, can be a major problem. The main reason is that the terrestrial environment acts as a sink for contaminants, due to its capacity for holding and retains pollutants, and due to long-term accumulation of contaminants the quality of soils may be negatively affected. As a result, historical sites in urban areas can be strongly impacted due to high levels of contaminants accumulated over years, being a good indicator of historical environmental pollution (Cachada et al., 2013; Liu et al., 2010a).

Soil can also act as source of contamination, depending on the controlling soil properties and contaminants themselves, through volatilization of contaminants, leaching to groundwater or runoffs to nearby aquatic ecosystems. It can also be a source through pathways such as the food web or direct ingestion, inhalation or dermal contact with soil by humans or animals. Soil, therefore, can have a direct effect on public health in addition to threats to ecosystems. Soil-generated dusts may also be a source of contamination and, due to their low particle size, the negative impacts on environment and human health can be enhanced both by chemical and physical effects. For example, in riverine or coastal areas, dusts can be transported to nearby aquatic systems resulting in an increase of the level of contaminants in sediments. Regarding human health, they tend to be more easily transferred through the aforementioned pathways (ingestion, inhalation and dermal contact). Yet, in urban areas, street dusts are formed not only by soil-derived material but also by deposition of airborne particles and other particles with different origin such as construction materials, garbage, weathered materials of pavements or automobiles (tire dust and debris, brake dust, body rust and tail pipe exhaust, diesel exhaust particles, etc). As a result, they can be a source of contamination themselves.

Hydrophobic organic contaminants (HOCs), such as polycyclic aromatic hydrocarbons (PAHs) or polychlorobiphenyls (PCBs), are a global environmental issue due to their carcinogenic and/or mutagenic potential, their massive use or continued emission, persistence and mobility throughout the environment (WHO, 2003). The long-range atmospheric transport turns HOCs into a transboundary environmental problem, reflected by their accumulation in soils all over the world, even at sites located far from human activity (Nam et al., 2009; Ravindra et al., 2008). HOCs present in urban soils are primarily a result of atmospheric fallout. Furthermore, despite the possibility of removal from soils through volatilization, photodegradation, leaching, fugitive dust, plant or animal absorption, and biodegradation, they can reach the soils again at a later moment in the same or different location. Thus, the loading of HOCs in soils is mostly a function of cumulative atmospheric deposition, minus losses due to volatilization, biodegradation and leaching. Once HOCs are in soils, they can also be incorporated into more stable solid phases over time, for instance, they can be retained in the organic phase of a soil, and this process known as aging, can be virtually irreversible (Ehlers and Luthy, 2003; Yang et al., 2001). Therefore, the contamination by HOCs is mainly a result of long-term pollution, causing chronic disruption of the biological processes within soil and having deleterious effects on ecosystems and jeopardizing human health. The lipophilic nature, hydrophobicity and low chemical and biological degradation

rates of HOCs leads to their bioconcentration and bioamplification, thereby potentially achieving toxicological relevant concentrations in organisms (Jones and de Voogt, 1999).

In the past, soil protection was usually addressed indirectly through measures to protect water and air, mostly because there was no awareness on its importance for ecosystems and economy. This perception comes as a result of the soils' great buffering capacities, slow reactions to contaminants and their natural spatial variability. In addition, the link between soil contamination and human health effects is not so evident as for water or air. In urban areas the soil quality assessment should aim to protect the soil community and nearby aquatic bodies, the protection of terrestrial vertebrates and human health. Indeed, nowadays several guideline values to regulate soil contamination have been developed in several countries (e.g. Jennings, 2012a), having as endpoint the protection of environmental health or aquatic environment, but most of them aiming the protection of human health (especially in residential areas). Yet, the availability of soil criteria is still somewhat deficient compared to criteria available for aquatic receptors (O'Halloran, 2006). One of the reasons for this is the lack of toxicological data for soil contamination, being most of guidelines based on aquatic ecotoxicological data (Antunes et al., 2010; Brand et al., 2013; Carlon, 2007). Other difficulties are related with the natural heterogeneity of soils and the difficulty of predicting the behaviour of contaminants in soils. For example, it is estimated to exist at least 10,000 types of different soils in Europe (EC, 2007).

The fate and behaviour of HOCs in soils is governed by different factors including soil properties (especially organic matter content), compound's properties, biota activity, ageing and environmental factors such as temperature and precipitation (Cachada et al., 2014; Reid et al., 2000a). It is now known that only a part of total concentration is available for partitioning between soil and solution and thus to be uptaken or transformed by organisms (bioacessible or bioavailable) or to be leached to groundwater (Alexander, 2000; Ehlers and Luthy, 2003; Semple et al., 2003). Therefore, the effects of contaminants to the environment and human health are related to their available fraction, rather than its total concentration, being very important to understand the sequestration and binding of HOCs in soil. Based on this, some European countries, such as The Netherlands and Spain, have already implemented a framework for the assessment of contaminated soils (environmental risk assessment plan) that comprises bioassays, residues analysis (soil and biota) and field monitoring (Fernández et al., 2006; Jensen and Mesman, 2006; Römbke et al., 2005). The aim of a risk assessment (RA) is to identify contaminants (hazards) and their likely occurrence (risks) in order to select priorities for environment protection and targets to control. It is a flexible approach that allows a tiered

fitness-for-use approach, i.e., sequential steps with increasing complexity as needed, being the principle "*simple if possible, complex when necessary*" (Swartjes et al., 2012). In this context a RA tool is any instrument that contributes to the determination of risks at a contaminated site (Swartjes et al., 2012). The tiered approach of a RA plan turn it in a cost-effective way of identify the potential risks to the environment and human health and to evaluate the resources required for remediation. A RA framework can be very useful in urban areas, since, due to the presence of multiple point and diffuse sources, it can be very difficult to assess the status of soil quality and the risks to environment and human health, without a systematic approach.

Typically, the first step for assessment of soil quality is based on the total content of contaminants in soil and its comparison with threshold values (usually named as soil quality guideline values or soil screening values) in order to perform a first calculation of potential risks. At this stage, multivariate and geostatistical tools can be very useful for site characterization, for example to evaluate the extent of contamination or to identify sources of contamination. Indeed, source apportioning is an important step that may help reducing the inputs of contaminants to soils. Assessing the total content is, however, a conservative approach since contaminants can be unavailable for uptake by organisms, therefore overestimating the risks. Higher steps of a RA plan are more site-specific and normally include the assessment of the available fraction. Since availability is compound and soil specific, particular assays to assess it are required. Traditionally this estimate is performed by using bioassays that respond to the bioavailable fraction, but in the last years, the chemical prediction of bioavailability has been a major issue, however not yet clarified (Cachada et al., 2014; Cui et al., 2013; Ehlers and Loibner, 2006). For example, it is known that one single chemical method could not predict availability for different organisms and the results depend on the compound as well (Kelsey et al., 1997; Ten Hulscher et al., 2003). Several chemical methods have been proposed and their potential to predict microbial mineralization is promising, but this will be very difficult to apply for higher organisms, due to the complexity of accumulation mechanisms which are not taken into account by chemical methods (Cachada et al., 2014). Still, chemical availability can be very useful to understand the bioavailability processes and the behaviour of HOCs in soils (e.g. mobility and leaching potential). Therefore, it has been suggested the inclusion of chemical availability in higher tiers of risk assessment together with bioassays (Brand et al., 2013; Jensen and Mesman, 2006). Even so, there are still some issues to be clarified regarding chemical availability, such as data interpretation or how to compare results with existing guidelines in order to perform a routine application and its incorporation in risk assessment.

1.2 OBJECTIVES AND HYPOTHESIS

The present work intends to be a comprehensive study conducted in two contrasting Portuguese urban areas (Lisbon and Viseu) by evaluating the levels of selected HOCs (PAHs and PCBs) in soils and their potential risks to human health and to the environment. In addition, it was aimed to understand the behaviour of these compounds in terrestrial environment and address their major sources of pollution. In order to achieve these goals, general properties of soils and levels of potentially toxic elements (PTEs) were also evaluated along with the application of multivariate statistics and geostatistical methods. Street dusts were also collected in an attempt to better understand sources of HOCs in urban areas and to evaluate the potential risks to the environment and human health posed by these contaminants in another environmentally relevant matrix.

The potential risks were evaluated by following a RA framework, which at a first stage was made by comparing total concentrations of contaminants with soil guideline values. Human health and environmental risks were also assessed by applying current approaches for risk calculation. The use of this methodology aimed to identify compounds and areas of concern, and to build risk maps using geostatistical tools. Since bioavailability should be included at a higher tier of RA and due to the attempts that have been made to predict bioavailability using chemical methods, these issues were discussed in detail. Because bioavailability is species and compound specific, a review of the processes involved on PAHs availability to microorganisms, earthworms and plants was performed and the outputs given by the different chemical methods were evaluated. Therefore, this study aimed at producing a state of the art knowledge base on bioavailability and chemical availability of PAHs in soils, clarifying which chemical methods can provide a better prediction of an organism exposure, and which are the most promising ones. The importance of incorporating chemical availability in risk assessment was also discussed. Finally, the second step of the RA plan included the study of the chemical availability (through a solid phase extraction method) and bioavailability (though bioaccumulation assays) of contaminants at selected sites. Therefore, the work intended to answer some questions:

1. Are PAHs and PCBs contaminants of potential concern in Lisbon and Viseu urban areas?
2. Which are the major inputs/sources of these contaminants?
3. Are the levels above guidelines representing some risk?
4. Is it possible to use chemical methods to estimate bioavailability?
5. Are the contaminants available or retained in soils, thus representing a lower risk?

1.3 STRUCTURE OF THE WORK

Chapter 2 presents the characteristics and environmental significance of selected HOCs (PAHs and PCBs) as well as a brief description of study areas. The selected risk assessment approach is also briefly explained, as well as its applicability herein. Finally, it is presented an overview of the most relevant guidelines regarding the presence of PAHs and PCBs in soils. Since higher tiers of risk assessment should include the assessment of bioavailability, Chapter 3 presents a review in order to understand what is known about the bioavailability of PAHs in soils and which potential chemical methods are available to predict it. In Chapter 4 a risk assessment model was applied to evaluate potential risks posed by the selected HOCs to the environment and human health in the two urban areas. This first screening allowed to identify PAHs in Lisbon soils as a potential environmental and human health issue. Based on the results of this first study, a new sampling campaign was conducted in Lisbon urban area in order to get a better insight on PAHs contamination. Further, in order to better understand the sources of PAHs, street dust samples were also collected, which allowed a classification of potential risks by using a more relevant matrix (Chapter 5). By using the advantageous large database obtained for Lisbon urban soils, it was possible to apply geostatistical methods, obtaining a better identification of areas of concern (risk maps) (Chapter 6).

Finally, this work intended to understand whether if the levels of PAHs in Lisbon urban soils are a real concern. As described in Chapter 3, it is not yet very clear how PAHs contamination affect the ecological receptors, due to physico-chemical factors affecting their availability. Moreover, based on results of this review it was decided to assess the PAHs mobility, and therefore the potential for leaching, by using a solid phase extraction method (Chapter 7) and assess their bioavailability by using bioaccumulation assays (Chapter 8). Chapter 9 presents the conclusions and final remarks.

Chapter 2

THE RISK ASSESSMENT APPROACH AND THE APPLICATION TO SELECTED ORGANIC POLLUTANTS IN THE STUDIED AREAS

2.1 GENERAL OVERVIEW OF THE RISK ASSESSMENT APPROACH

There are two major approaches of risk assessment (RA): predictive and retrospective (or diagnostic). The predictive RA is associated with the authorization and handling of hazardous substances in order to regulate their use and prevent the introduction onto the market and, consequently, their environmental release (Jensen et al., 2006; van Gestel, 2012). This approach uses laboratory toxicity data (or controlled and manipulated semi field studies) to extrapolate to real-world situations and derive thresholds or safe levels of chemicals in the environment. In Europe, the existing RA plans were mostly based on the European Union Technical Guidance Document (EU-TGD) on new notified substances, existing substances and on biocidal active substances or a substance of concern present in a biocidal product (EC, 2003).

The retrospective (or diagnostic) RA approach consists in the assessment of damages or effects caused by contamination at a given site (Jensen et al., 2006; van Gestel, 2012). It allows the identification of the chemicals of potential concern, the exposure of humans and ecological receptors and the risks resulting from such exposures. Consequently, diagnostic RA will select priorities for environment protection, risk reduction and remediation (van Gestel, 2012). Indeed, this approach has been considered the most cost-effective scientific tool for managing contaminated sites (Swartjes et al., 2012).

In both approaches the framework can be conducted in order to protect human health, ecosystems, and groundwater tables or nearby freshwater bodies; hence, it always includes a source-pathway-receptor pollutant linkage. The evaluation is based on physical, mathematical and statistical models, providing an estimation of risks, i.e. the probability of an adverse effect occur as a result of an exposure to a substance or a mixture of substances (Suter, 2006).

The United States superfund program is the most developed diagnostic RA model, since it was also one of the firsts to be developed. Yet, several other countries (e.g. Netherlands, Canada, UK, Spain) have already developed their own RA plans. Due to differences observed between countries (Carlon, 2007; Provoost et al., 2006), in 2005 the European Commission Joint Research Centre initiated a network to promote the development of common risk assessment tools for contaminated soils called “Human and Ecological Risk Assessment for Contaminated Land in European Member States” (HERACLES) (Carlon, 2007). Nevertheless, the harmonization of methodologies was not yet possible (Swartjes et al., 2008). Even so, most of the RA models are based on the same generic framework (Figure 2.1) which is known as the “universal risk assessment paradigm” (Jensen and Mesman, 2006; Posthuma et al., 2008; Weeks and Comber, 2005).

Typical, RA starts by an initial problem definition or hazard identification, based on a preliminary site characterization and includes the purpose of the assessment, followed by a plan for analyzing and characterizing risks. At this point it is built a conceptual site model, that includes the potential sources of contamination (qualitative/quantitative; primary/secondary), the identification of contaminants of potential concern, and assessment endpoints (USEPA, 1991,1998). This first stage normally includes the information about soil characteristics, past and future land-use (e.g. industrial, residential, agricultural or natural areas), with the former deciding all the decision process. The assessment and the measurement endpoints comprise the receptors and the effects to assess, respectively. After identifying the receptors, the potential exposure pathways are defined, i.e. the way a contaminant comes in contact with an organism (e.g. by ingestion, inhalation or dermal contact). In order to understand the link between source-pathway-receptor, it's necessary to study the environmental behaviour of contaminants and therefore it is important to collect data for modelling contamination fate and transport.

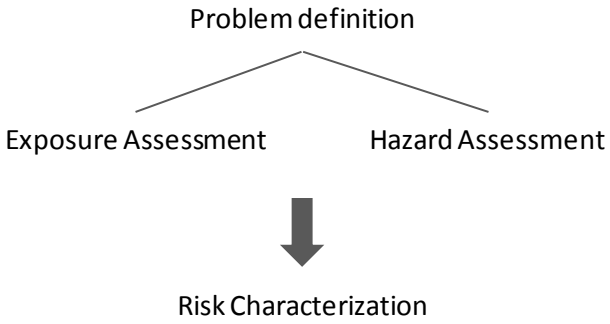

Figure 2.1 - Generic risk assessment framework.

The further step of RA is the hazard (or effect) assessment together with the assessment of the exposure of receptors to contaminants. In order to proceed to the hazard or toxicity assessment, which includes hazard identification and characterization it is needed to collect information about the toxicity/ecotoxicity of contaminants to estimate for example the tolerable daily intake or the predicted effect concentration. This step is therefore related to the intrinsic properties of contaminants. The exposure or dose assessment quantifies the magnitude and spatial and temporal distributions of exposure for the scenarios developed during problem formulation and therefore it is related to the intensity, frequency, and duration of actual or hypothetical exposure. For example, it estimates the intakes based on the routes of exposure, such for example the soil ingestion rate, and which is the response to the dose.

Finally, the integration of results obtained in the exposure assessment together with the hazard assessment (regarding human or environmental health) allows performing a risk characterization, and finally a risk management can be conducted. In other words, risk characterization gives an estimate of risks by combining expected exposure levels and expected effects. Hence, risk calculation should include a quantification of the likelihood and a characterisation of the extent of effects. Yet, it should be kept in mind that risk only exists if the three components of the linkage source-pathway-receptor are present. This step includes the uncertainty, assumptions, and scientific judgments of the previous ones.

In most European countries, the RA of contaminated soils consists in a tiered approach that generates data in sequential steps with increasing complexity (Jensen and Mesman, 2006; Swartjes et al., 2012; Tarazona et al., 2005; Weeks and Comber, 2005). Typically, at a first tier of RA the evaluation of risks are based in simple hazard quotients (HQ), by comparison with toxicological data or generic soil quality guidelines (EC, 2003; Solomon and Takacs, 2002; Tarazona et al., 2005; Zolezzi et al., 2005). This approach is followed by several European countries such as The Netherlands, UK, Denmark, Finland or Spain (Carlon, 2007; DEPA, 2002; Tarazona et al., 2005). For example, in the particular case of The Netherlands the assessment of contaminated sites is first determined by comparison of measured total concentrations with soil quality guidelines (Jensen et al., 2006). It is recommended moving to the next tier (typically a site-specific RA) if levels are exceeded, i.e., only if soil contamination is observed, and the decision takes into account the dimensions of the impacted area (Swartjes et al., 2012). This simple comparison with guidelines may underestimate or overestimate risks since: a) guidelines or toxicity values may be derived from freshly spiked soils (meaning that the contaminants may be totally available); b) normally these values are derived for single chemicals using single species toxicity data, no mixture effects or ecological interactions were taken into account; c) in some cases soil properties were not taken into account (O'Halloran, 2006). Even being very conservative, this is a simple and easy approach to identify substances that can be a threat and areas that need a more detailed risk assessment.

As referred previously, the risk assessment can be performed for human health, ecosystem or groundwater, depending on the receptors that were identified in the problem definition step, and in the next tiers, the refinement is made according to the specificities of the receptors. At these higher stages probabilistic procedures, in which the likelihood of exposure and effect are considered, are typically used for assessment of risks (Solomon and Takacs, 2002; Zolezzi et al., 2005). The complexity of data increases as one move through the tiers, and in each one a more

detailed site-specific analysis is needed. Therefore, procedures will be related to the specific land use and new data may be needed since soil use will define receptors and exposure pathways. At this stages site uses can be subdivided into levels of sensitivity (e.g. land uses where there is an evident likelihood of children ingesting soil or areas of natural beauty where sensitive species are present can both be considered very sensitive). The collection of more detailed data on environmental concentrations, fate and transport may also be needed. It is advised to move to the next tier only if results from the previous tier are not satisfactory.

One of the major problems of risk assessment are the large uncertainties, particularly for ecological receptors, for example due to problems in the inclusion of bioavailability or due to the lack of validation of transfer and exposure models (Carlon, 2007). Yet, these uncertainties become smaller as moving though the tiers. Other problems are that soil systems are very heterogeneous being this especially true in urban areas due to their patchy nature.

2.2 GENERAL CHARACTERIZATION OF THE STUDY AREAS

Two contrasting Portuguese urban areas (Lisbon and Viseu, which differ in geological and climatic conditions, industrial and urban development processes) were studied (Figure 2.2). Lisbon city (Figure 2.2a) is the largest urban area of Portugal, with a population of 547,631 inhabitants and an area of 85 km^2. The Lisbon metropolitan area is highly industrialized (petrochemical, chemical, textile, shipyard and metal processing industries), although most of the industry is located outside the urban area. A harbour is located inside the urban area, which is one of the most touristic harbours in Europe and has a very high commercial importance; there's also the international airport, which is the largest in the country. In addition, several highways cross the city, with approximately 170,000 cars entering the city every day. Even though the carpool of Lisbon metropolitan area is not very old (36% of cars are more than ten years old), traffic is one of the most important sources of pollution. The south-western area of the city is occupied by one of the biggest urban parks of the Europe (Monsanto Park), with an area of almost 10 km^2. Geologically, Lisbon area is composed by a great variety of rocks: lava flows (Lisbon Vulcanic Complex rocks) and limestone at south-west; sandstones with calcic intercalations and terraces are predominant in the rest of the area. Above these rocks, which constitute the substrate of the territory, it is possible to find recent deposits, such as sand dunes and beaches (all with calcic nature), as well as the alluvial plain formed by several watercourses including the Tagus River.

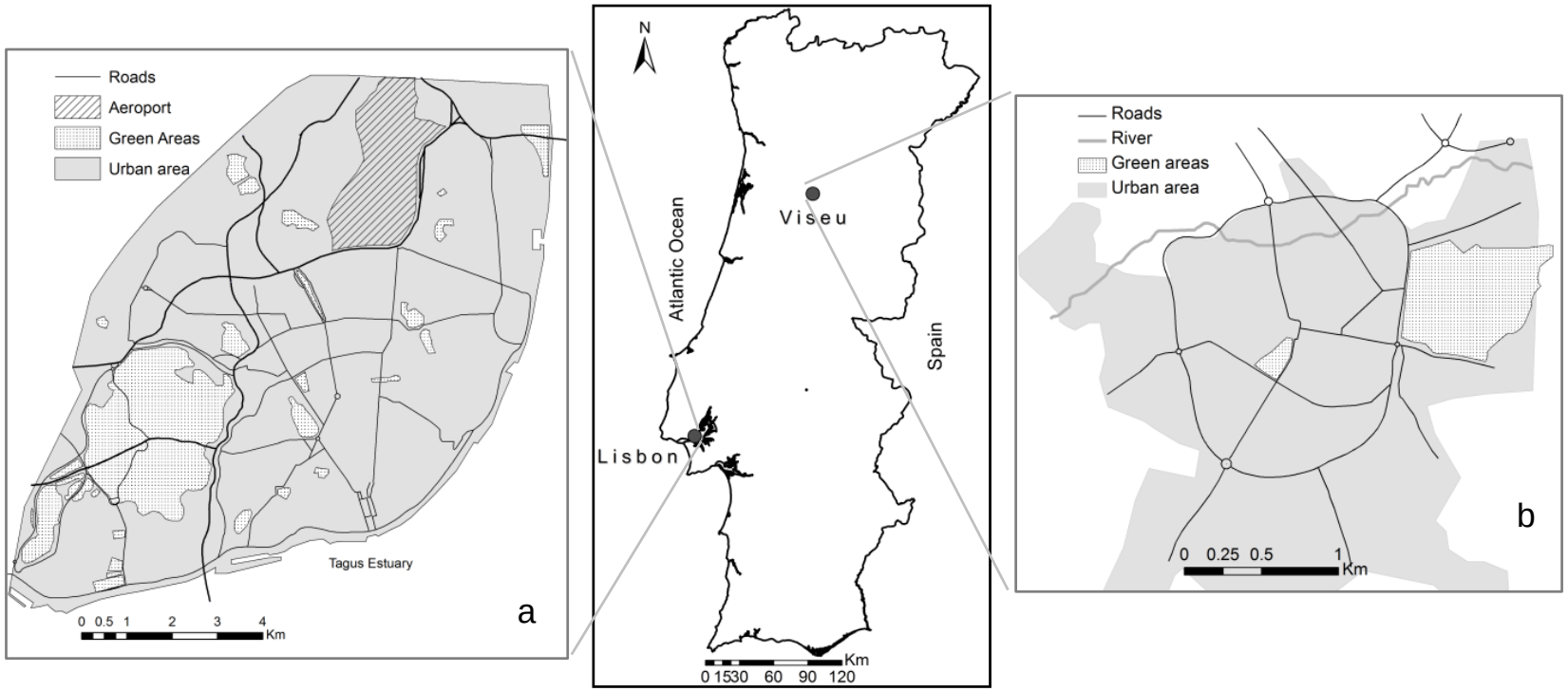

Figure 2.2 - Location of the studied urban areas: Lisbon (a) and Viseu (b).

Soils are mainly chromic vertisols at south-weast, gleyic solonchaks at north-east and calcic cambisols in the rest of the area, according to the FAO classification[1;2]. The climate in the Lisbon region is temperate, with an average daily temperature of 17.5°C, being the temperature in the town centre normally 2°C higher, especially during summer. The total annual rainfall is 700 mm.

Viseu (Figure 2.2b) is a small city with 47,250 inhabitants and the urban centre has around 12 km^2. Industrial activities are present at a small scale, being the most important economic activities related with catering industry, wholesale and retail sectors. Regarding traffic, cars are the main transport used and the city is located nearby (around 3 km) one of the most important highways of the country. Viseu is in the Iberian Central Zone where the predominant rocks are mainly schists and granites. Soils are classified as humic cambisols (associated to dystric cambisols) from eruptive rocks. The climate in Viseu is characterized by temperature extremes, with rigorous and wet winters and hot and dry summers. The daily mean temperature is 15°C, and the total annual rainfall is 1400 mm.

Two major groups of contaminants are normally found at potentially hazard levels in urban soils (potential toxic elements and organic contaminants). Typically, the potential toxic elements (PTEs) of concern include Cu, Hg, Pb or Zn and the organic contaminants include polycyclic aromatic hydrocarbons (PAHs), polychlorobiphenyls (PCBs) or pesticides (Cachada et al., 2013; 2009; 2012b). The origin of contamination can be anthropogenic (e.g. traffic or industry) or telluric (e.g. local geology or natural fires) and a generic conceptual model for urban areas can be built as shown in Figure 2.3. The pathway of contaminants into soils is mainly atmospheric deposition, but also dusts (from pavements, tires or brakes), leaks from automobiles or direct disposal may also contribute. However, runoff from roads into soils and vegetation deposition may also be considered. Once in soils contaminants may be re-circulated by re-volatilization, leached through the water solution or taken by biota. Dusts either formed by soil derived material or by deposition of particles can be transported outside urban areas or to nearby aquatic systems. Therefore, the receptors are the aquatic bodies, plants, soil organisms, vertebrates and humans. The pathways are the inhalation, ingestion and dermal contact of soil or dusts. In urban areas there is a variety of land uses, with different patterns of source-pathway-receptor, such as roadsides, residential areas, schools/kindergartens, gardens and parks.

[1] Food and Agriculture Organization of the United Nations (http://www.fao.org/soils-portal/soil-survey/soil-classification/en/)
[2] Agência Portuguesa do Ambiente. Atlas do Ambiente (http://sniamb.apambiente.pt/webatlas/)

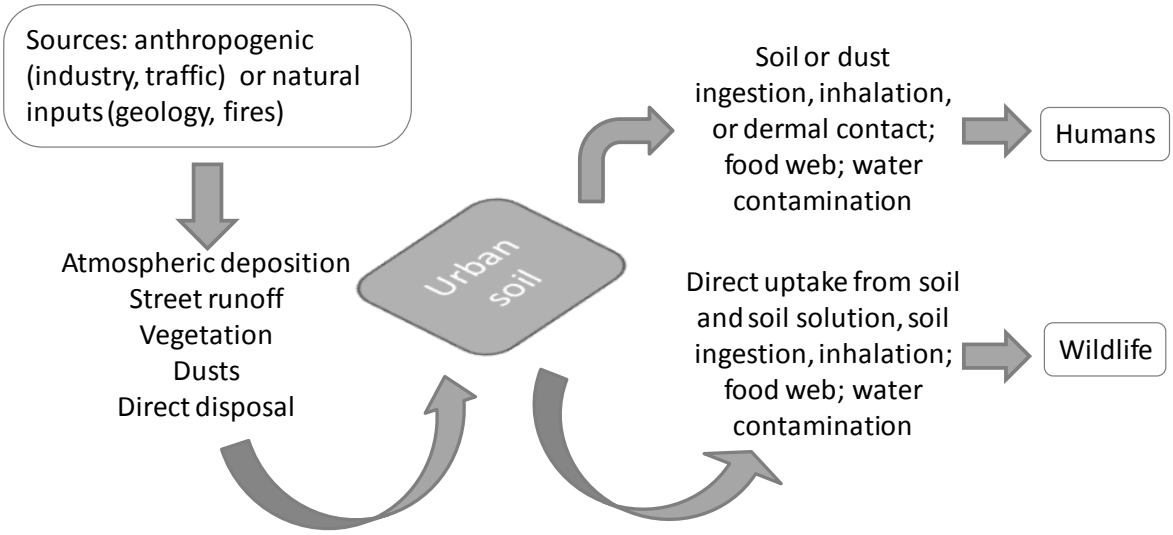

Figure 2.3 - Generic conceptual model for urban soils risk assessment.

2.3 CHARACTERISTICS AND ENVIRONMENTAL SIGNIFICANCE OF SELECTED CONTAMINANTS

This study is mainly focused on organic contaminants and, within them, two groups were selected: PAHs and PCBs. The reason for this selection was because they are semi-volatile, chemically stable and hydrophobic organic compounds which are ubiquitous in the environment and good markers of urban activities (Fabietti et al., 2010; Ma et al., 2009; Zhang et al., 2007). The importance and interest of studying these groups of contaminants is related to their tendency to accumulate in soils, where they can persist for several years, and to the bioaccumulation potential. In addition, their environmental significance is also related to their detrimental biological effects caused by their toxicity, carcinogenic, mutagenic and/or endocrine disrupting properties.

PAHs are planar molecules composed only by carbon and hydrogen, and they have two or more fused aromatic rings in their structure. It's a large group of compounds consisting of more than one hundred individual homologues and isomers. Yet, this study is focused on the determination of 16 priority PAHs defined by the US EPA (Table 1 of Annex I): acenaphthene (ACE), acenaphthylene (ACY), anthracene (ANT), benzo(a)anthracene (BAA), benzo(b)fluoranthene (BBF), benzo(k)fluoranthene (BKF), benzo(ghi)perylene (BGHI), benzo(a)pyrene (BAP), chrysene (CRY), dibenzo(ah)anthracene (DBAH), fluoranthene (FLA), fluorene (FLU), indeno(1,2,3-cd)pyrene (IND), naphthalene (NP), phenanthrene (PHE) and pyrene (PYR). These 16 compounds are also considered surrogates of the larger suite of PAHs (Kapustka, 2004). They are by-products primarily from the incomplete combustion or pyrolysis of organic material, and therefore the origin could be either natural or anthropogenic. However, anthropogenic activities such as traffic, industry,

domestic heating and incineration processes are major sources of these compounds into the urban environment, mainly through atmospheric emission. PAHs reach the urban soils mostly from fallouts of particulate material suspended in the atmosphere, adsorption of gaseous form PAHs or street dusts. PAHs may also be formed by the slow maturation of organic matter and as a consequence petroleum and natural gas are also enriched in these compounds (petrogenic origin). PAHs are among the most widespread contaminants found in soils.

PCBs are chlorinated hydrocarbons with a biphenyl nucleus on which one to ten of the hydrogens have been replaced by chlorine (C_{12} $H_{10-n}Cl_n$) (Figure 1 and Table 2 of Annex I). As a result, this group encompass 209 congeners, with different chemical and biological properties that depend on the number and arrangement of chlorine atoms. However, this study is focused on 21 PCBs congeners with the following IUPAC number: 1, 5, 18, 28, 31, 44, 52, 66, 87, 101, 110, 118, 138, 141, 151, 153, 170, 180, 183, 187 and 206. The choice of these congeners was based on both the USEPA and the Dutch lists and represents the common Arochlor formulations which is the trade name of commercial complex mixtures most used in Europe. Among these congeners, 7 are considered indicators and are the ones normally referred in the guidelines: 28, 52,101, 118, 138, 153 and 180. PCBs cannot be formed in nature and its usage is related with industry, even though nowadays their production is ceased. Yet, they are still present in many products (e.g. capacitors, transformers, hydraulic fluids, cutting oils, etc.) and they can also be formed as by-products of certain industrial processes or waste incineration (EA, 2007). Therefore, the sources of these compounds to urban soils can be both atmospheric deposition and accidental disposal.

2.4 ENVIRONMENTAL RISK ASSESSMENT (ERA)

Environmental risk assessment (ERA) can be defined as a "*process that evaluates the likelihood that adverse ecological effects may occur or are occurring as a result of exposure to one or more stressors*" (USEPA, 1992,1998). The application of ERA can be very complex, since it includes multiple species and intrinsic characteristics of individuals, populations, communities, and ecosystems. In practice it includes collecting, organizing and analysing environmental data in order to estimate the risk of contaminants to ecosystems by using models of exposure representative of target species (Jensen et al., 2006). Recently, the TRIAD concept has been suggested as a cost-effective approach to be included in the harmonisation process of ERA for soil contamination in Europe (Dagnino et al., 2008; Jensen and Mesman, 2006; Swartjes et al., 2008). This TRIAD approach is also based on the tiered system, but decisions at each level are supported by combining evidences from three different scientific lines of evidence (chemical,

ecotoxicological and ecological). However, in the present work this approach will not be used since ecological data was not collected.

The four typical tiers in ERA are: Tier 1 - Simple screening; Tier 2 - Refined screening; Tier 3 - Detailed screening; Tier 4 - Final assessment (Jensen and Mesman, 2006). At the screening level (tier 1) assessment is mostly based on hazard quotients (HQs) which consists in the comparison of the exposure concentration, or the environmental concentrations (EC), with the acceptable level of effect (for example, soil screening guidelines) as shown in Eq. 2.1 (Arnot et al., 2011; Backhaus et al., 2010).

Eq. 2.1

$$HQ = \frac{\text{EC}}{\text{Acceptable level}}$$

Instead of the acceptable level of effect it can also be used an estimated no-effect assessment concentration obtained from laboratory bioassays, such as the predicted no effect concentration (PNEC) as shown in Eq. 2.2 (Zolezzi et al., 2005). The PNEC is defined as "*the concentration below which unacceptable effects on organisms will most likely not occur*", and in this case the ratio is called Risk Characterization Ratio or Toxic Unit (TU) (EC, 2003; EU, 2008; Zolezzi et al., 2005).

Eq. 2.2

$$TU = \frac{\text{EC}}{\text{PNEC}}$$

Considering the aforementioned equations, it is clear that the definition of risks is extremely related to the definition of the assessment endpoint. For example, when using the PNEC, a ratio higher than 1 indicate that unacceptable effects on organisms are likely to occur, yet, whether this ration gives or not a characteristic of the extent of effects will depend on the method used to calculate the PNEC (EC, 2003). PNEC can be calculated using ecotoxicity data, normally using the non observed effect concentration (NOEC) of the most sensitive species combined with an assessment factor, or, if test data are lacking, the equilibrium partitioning method can be used (EU, 2008; Verbruggen, 2012). In both cases it is not provided any quantification of the expected impact. Other toxicological endpoints can be used in the TU model, either acute (e.g. median lethal concentration - LC50) or chronic (e.g. long-term NOEC). Using the PNEC value is a very conservative approach, since it is derived by applying an assessment factor to ecotoxicological

endpoints obtained for the most sensitive organism, which may be different for different chemicals. This deterministic approach is, therefore, adequate for identification of possible impacts and for prioritisation (SCCS et al., 2012). In the present work, TU were calculated only for PAHs using the PNEC values given in Table 2.1, since there are no PNEC values for PCBs. These PNEC values were taken from the EU risk assessment report for coal-tar pitch (EU, 2008).

In the risk assessment report for coal-tar pitch from the EU (EU, 2008) it is advised to sum the individuals TUs of the 16 PAHs, obtaining the toxicity of the mixture (TUm). This approach assume that individual compounds have the same toxic mode of action (TMoA), and in the particular case of PAHs it is caused by narcosis, being possible to apply the concentration addition model (CAM) (de Zwart and Posthuma, 2005; EU, 2008; SCCS et al., 2012; Verbruggen, 2012). The exposure to the mixture is considered a risk if the sum is higher than 1. Since environmental quality guidelines are normally derived by extrapolation from ecotoxicological endpoints (e.g. by applying assessment factors), the definition of TU is conceptually comparable to the HQ, being the TUm equivalent to Hazard Index (HI), which is the sum of individual HQ.

PNEC values may also be calculated by using the Species Sensitivity Distribution (SSD) curves. The approach consists in plotting ecotoxicity data from laboratory tests with different species, obtaining a cumulative probability density function (Swartjes et al., 2012). In this case PNEC is normally calculated as being the 5th percentile of a SSD based on chronic NOECs, and the EC/PNEC ratio (Eq. 2.2), together with the slope of the SSD, gives a quantification of the likelihood and a characteristic of the extent of effects. If EC is equal to PNEC (EC/PNEC=1) this means that only 5% of the species are expected to be affected. The levels taken from the SSD are normally known as hazard concentrations (HCx) at which a certain percentage of all species is assumed to be affected. For example, if the 5th percentile is considered, the PNEC value will correspond to an HC_5. The SSD approach is used for example by the Ecological Risk Assessment tool in Netherlands, together with the Soil Quality Triad (Swartjes et al., 2012). However, in the particular case of PAHs and PCBs there is a deep lack of soil ecotoxicity data to obtain these distributions for individual compounds. In order to overcome this constrains, the Dutch authorities (through RIVM) recently presented a method to derive risk limits for PAHs in soil by using organism's internal residues (including aquatic, benthic and terrestrial species) to obtain a SSD model (Verbruggen, 2012). The approach consists in first calculate pore water concentrations by considering partitioning between organic carbon and water, and then calculate the internal residues from water concentrations using a partition coefficient. It is assumed that: a) the effects of the 16 PAHs occur at the same concentration in aquatic or terrestrial organisms; b) uptake occurs only via pore water; c) the

CAM can be applied. Considering this it was possible to derive an HC_5 for each individual compound and, by applying an assessment factor of 5 to account for uncertainties, a maximum permissible concentration (MPC) was calculated (Table 2.1). If comparing this MPC values with the PNEC values (normally derived from the NOEC values of the most sensitive species combined with an assessment factor) shown in Table 2.1, it is clear that the former are influenced by the physico-chemical properties of individual compounds. In spite of the standardization of values for different soil properties, it is clear that while for PNEC there is no relationship between this value and the molecular weight, the MPC (HC_5) increases with the molecular weight (together with the increase in hydrophobicity). These differences are clearer if looking to the serious risk concentration (SRC) values presented in Table 2.1, also calculated using two different approaches and standardized for the same soil properties. In the particular case of the SRC obtained from the SSD curve (chronic NOECs recalculated for organism's internal residues), it corresponds to an HC_{50}.

Table 2.1 - PNEC values of PAHs for soil organisms and MPC and SRC's concentration (all in µg kg^{-1}) calculated using different methods (EU, 2008; Verbruggen, 2012).

Compound	PNEC[a]	MPC (HC_5)[b; c]	SRC[b; d]	SRC (HC_{50})[b; c]
NP	1,000	430	14,000	26,000
ACY	290	510	9,400	30,000
ACE	38	530	31,000	31,000
FLU	1,000	580	82,000	35,000
PHE	1,800	670	90,000	40,000
ANT	130	710	60,000	42,000
FLA	1,500	990	309,000	59,000
PYR	1,000	890	53,000	53,000
BAA	79	1,900	91,000	112,000
CRY	550	1,700	38,000	103,000
BBF	280	2,600	62,000	153,000
BKF	270	2,500	44,000	151,000
BAP	53	2,600	76,000	154,000
IND	130	4,900	89,000	289,000
DBAH	54	4,700	18,000	279,000
BGHI	170	3,100	10,000	186,000

[a]standard soil with 2% OC and 3.4% OM; deterministic approach in which values are derived either from ecotoxicity tests using an assessment factor or by EqPT; [b]standard soil with 10% OM; [c]calculated using internal residues and the SSDs approach; [d]deterministic approach in which values are calculated from a geometric mean of ecotoxicity endpoints and/or by EqPT.

The Dutch methodology has a standard protocol in which cumulative risks of mixtures in one sample are expressed as toxic pressure (TP) (de Zwart and Posthuma, 2005; Swartjes et al., 2012). The TP can be calculated using two approaches depending on the ecotoxicological data used: one for single-species effect prediction and the other for species assemblages. The first case applies to data obtained by testing each compound with the species under evaluation (NOECs, lowest observed effect concentration - LOECs, or effect concentration - ECx) which is used to compare with actual exposure concentrations giving the toxic equivalent quotients. For compounds with the same TMoA these toxic equivalent quotients can be added, following the CAM approach, and the sum of these quotients is called toxic units (TU), as shown in the following equation (Eq. 2.3):

Eq. 2.3

$$\mathrm{TU_{TMoA}} = \sum \frac{d(A)}{D(A)} + \frac{\mathrm{d(B)}}{D(B)} + \cdots + \cdots$$

where d(A) and d(B) is the actual exposure concentrations of the compounds A and B; and D(A) and D(B) is the exposure concentrations of A and B that cause a standard response, such as a LC50, EC50 or NOEC. The individual terms d(A)/D(A) and d(B)/D(B) are called toxic equivalence quotients (TEQ). The TP for compounds with the same TMoA (TP_{TMoA}) are then calculated using Eq. 2.4. But considering that within a complex mixture, we can find groups of compounds with the same TMoA within the group, but with different TMoA between groups, the toxicities of different modes of action (TP_m) can be combined by using the response addition model (RAM) (Eq. 2.5).

Eq. 2.4

$$TP_{TMoA} = \frac{1}{1 + e^{-log(TU_{TMoA})\beta_i^{-1}}}$$

Eq. 2.5

$$TP_m = 1 - (1 - TP_{TMoAi})(1 - TP_{TMoAj}).....(1 - TP_{TMoAn}) = 1 - \prod (1 - TP_n)$$

The second approach to calculate the TP is for species assemblages and uses effect concentrations calculated from SSDs. Thus, ecotoxicological data for each compound and for a battery of species is integrated in SSDs from which hazard concentrations for a given percentage of species are obtained (for example an HC_5), and these HC_x replace the single point estimates mentioned above in the calculation of TP. Afterwards, the application of the CAM and RAM models follow the same approach but this time providing data about the risks of the mixture for a

potential fraction of species rather than for a single-species. Therefore, instead of a TU it will be calculated the Hazard Unit (HU), which is given by the ratio between the concentration of contaminant (EC) and the toxicity threshold taken from the SSDs. Considering the CAM, the HU for compounds with the same TMoA (HU_{TMOA}) can be added as shown in Eq. 2.6. Hence, the output of this model (Eq. 2.7) can be defined as the potentially affected fraction (PAF) since it allows quantifying the fraction of species that is expected to be locally affected beyond the selected effect level. Once the PAF value is calculated considering the sum of HU_{TMoA}, it can be called the multisubstance PAF (mSPAF). Further, if the mixture is composed by groups of substances with different modes of action among them, the RAM model can also be applied through the equation Eq. 2.8.

Eq. 2.6

$$\mathrm{HU_{TMoA}} = \frac{\mathrm{EC_1}}{\mathrm{HCx_1}} + \frac{\mathrm{EC_2}}{\mathrm{HCx_2}} \ldots + \frac{\mathrm{EC_n}}{\mathrm{HCx_n}}$$

Eq. 2.7

$$PAF_{TMoA} = \frac{1}{1 + e^{-log(\sum HU_{TMoA})\beta_{TMoA}^{-1}}}$$

Eq. 2.8

$$mSPAF_{RA} = 1 - \prod (1 - PAF_{TMOA})$$

In the present study it were used the MPC (corresponding to an HC_5) and the SRC (corresponding to an HC_{50}) values shown in Table 2.1, calculated using internal residues and the SSDs approach as suggested by Verbruggen (2012). Considering the narcosis effect of PAHs, the CAM will be used and the individual HU for compounds will be added. Therefore, the combined toxicity for a group of contaminants with a comparable mode of action (PAF_{TMoA}) will be calculated using the Eq. 2.7 (de Zwart and Posthuma, 2005; Jensen and Mesman, 2006; Swartjes et al., 2012). Since the β value was not provided by Verbruggen (2012) the default slope value of 0.4 will be used. Since some authors suggest that toxicity of PAHs would be better represented by RAM, this approach was also considered (de Zwart and Posthuma, 2005; Olmstead and LeBlanc, 2005). Therefore, the PAF_{TMoA} was calculated individually for each compound using Eq. 2.7 and the $mSPAF_{RA}$ was calculated as shown in Eq. 2.8

Jensen and Mesman (2006) propose a methodology to calculate the TP for an area contaminated with PAHs that uses soil screening levels. Even that is normally considered the same mode of action for PAHs, being assumed that toxicity of all PAHs is similar and caused by narcosis

(EU, 2008; Posthuma and de Zwart, 2012; Verbruggen, 2012), these authors assumed a dissimilar toxic mode of action for all compounds. Further, these authors followed the equations described above to calculate the risks for species assemblages, with some adjustments since there are no SSDs available for individual PAHs based on ecotoxicological data. Therefore, they suggested to calculate the HU for each individual compound by using soil screening levels (which were obtained with assessment factors) and a default slope value (β=0.4) as shown in Eq. 2.7, but in this case it will be named TP instead of PAF (Jensen and Mesman, 2006). Considering this, the TP of the complete mixture of contaminants (TP_m) is calculated using the RAM as shown in Eq. 2.8 (de Zwart and Posthuma, 2005; Jensen and Mesman, 2006).

2.5 HUMAN HEALTH RISK ASSESSMENT (HHRA)

The aim of human health risk assessment (HHRA) is the identification of the potential for adverse health effects on humans that can be caused by exposures to environmental hazards. The general framework is the same shown in Figure 2.1, in which estimates of environmental exposure are combined with the known adverse effects of exposure to obtain an overall estimate of the potential public health risk. Specifically, the first step, hazard identification or problem definition, includes the identification of physiological effects/health problems caused by the pollutants and the determination of whether exposure to those pollutants can cause an increased incidence of such adverse health effect (e.g. cancer or birth defects). The second step includes the hazard assessment and the exposure or dose-response assessment. It consists in the characterization of the health problems and the different physiological effects occurring at different exposures and it should consider factors that could affect susceptibility, such as intensity and patterns of exposure, age and lifestyle variables. The exposure assessment, in addition to generic steps (source and pathways identification; contaminant concentration; exposure scenarios, pathways and routes of exposure), considers exposure factors related to human behaviours that define intensity, time, frequency, and duration of actual or hypothetical exposure as well as identifying the extension of the exposed population (USEPA, 2011a). The dose-response assessment can be calculated by using two approaches: the threshold approach or a non-threshold approach. The first is based on the principle that there is a range of exposures from zero to a value that can be tolerated without adverse effects, i.e., a certain threshold needs to be exceeded before a toxic effect will occur. In the second case compounds are assumed to exert their activity also at the smallest dose, i.e., theoretically it's not possible to define a level of exposure with a probability of generating

response and, consequently, there are no threshold values for such compounds. This is the case of genotoxic carcinogens and it's normally referred as a "linear" dose-response assessment.

Risks result from the combination between exposure and toxicity and, in a simplistic way, they can be characterized just by comparing the estimated daily intake (ingested, inhaled or to absorbed) with the estimated tolerable daily intake (TDI) (Carlon, 2007). However, the exposure assessment can be complex due to site or population specific conditions. Therefore, an average daily dose (ADD) can be estimated. It consists in the average of exposures or doses over a period of time as shown in Eq. 2.9, being Bw the body weight and AT the averaging time (USEPA, 2011a).

Eq. 2.9

$$ADD = \frac{\text{Intake dose}}{\text{Bw} \times \text{AT}}$$

The intake dose is calculated as shown in Eq. 2.10, where C is the concentration (mass or volume), IR is the intake rate (mass/time) and ED is the exposure duration (time). For acute exposures the dose can be averaged over a day or a single event, whereas for cancer exposure the duration is always the lifetime (normally 70 years). Hence, several estimations need to be made, for example the characterization of individuals (body weight, lifetime, etc), and exposure patterns (soil ingestion or inhalation rate, exposure frequency, etc...).

Eq. 2.10

$$\text{Intake dose} = \text{C} \times \text{IR} \times \text{ED}$$

One of the most developed HHRA programs is the one developed by USEPA (named IRIS[1]), that provides the scientific support for USEPA risk management decisions since it includes information that can be used to support the hazard identification and dose-response assessment steps. In Europe, one of the most used models is the one from The Netherlands, in which the hazard assessment includes dose-response assessment, resulting in the Critical Exposure Value (CEV) for a contaminant. The human exposure (result of exposure assessment) is then tested against the CEV leading to risk characterization (Swartjes et al., 2012).

[1] http://www.epa.gov/iris/

2.5.1 No cancer effects

Oral reference doses (RfD_O) and inhalation reference concentrations (RfC), were developed for the non-carcinogenic effects of substances, known or assumed to be produced through a nonlinear (possibly threshold) mode of action. The RfD_O (expressed in mg of substance/kg body weight-day) is defined as an estimate of a daily exposure (including sensitive subgroups) that is not likely to result in an appreciable risk of deleterious effects during a lifetime. A RfD_O can be derived from an overall no-observed-adverse-effect level or lowest-observed-adverse-effect level, available from toxicity studies, and then divided by an assessment factor (Swartjes et al., 2012). A benchmark dose can also be used, which is derived from statistical regression analysis. The inhalation RfC (expressed in mg of substance/m^3) is analogous to the oral RfD_O but provides a continuous inhalation exposure estimate. In the present study it will be used whenever data is available for inhalation of particles since concentrations in air were not monitored.

Human health risk assessment on a chronic basis can be performed by calculating the non-cancer hazard quotient (HQ), which is given by the ratio between the predicted exposure (Exp) to a contaminant over a specified time period and the oral reference dose (RfD_O), as shown in Eq. 2.11 (USEPA, 1991).

Eq. 2.11

$$HQ = \frac{Exp}{\mathrm{RfD_O}}$$

In the present work the HQ associated with each compound exposure, was calculated based on the deterministic approach from USEPA RAGS methodology (USEPA, 1991). The potential exposure to the amount of contaminants contained in residential soil was calculated as described in the USEPA screening levels equations for preliminary remediation goals (USEPA, 1991,2011b). Therefore, exposure was calculated separately for each potential route, ingestion (Exp_{ing}) dermal absorption (Exp_{der}), and inhalation of particulates (Exp_{inh}), as shown in Eq. 2.12, Eq. 2.13 and Eq. 2.14, respectively; where C_{soil} is the concentration in soil and the definition of the variables used in the equations can be found in Table 2.2. The potential exposure was calculated separately for children and adults. All exposure parameters, i.e. variables used for the calculation of intake such as exposure duration or average body weight, were first selected according to EPA's default values (Table 2.2). Values proposed by other institutions are also presented in Table 2.2.

Compound specific parameters used in health risk assessment for the two groups of compounds are presented in Table 2.3.

Eq. 2.12

$$Exp_{ing} = \frac{C_{soil} \times EF \times ED \times IRS}{AT \times Bw \times 10^6} \ (mg\ kg^{-1}\ d^{-1})$$

Eq. 2.13

$$Exp_{der} = \frac{C_{soil} \times EF \times ED \times SA \times AF \times ABS}{AT \times Bw \times 10^6} (mg\ kg^{-1}\ d^{-1})$$

Eq. 2.14

$$Exp_{inh} = \frac{C_{soil} \times EF \times ED \times ET}{AT \times PEF} \ (mg\ m^{-3})$$

The non-cancer hazard quotient for ingestion (HQ_{ing}) is calculated by dividing the potential oral exposure by the RfD_O (Eq. 2.15). The non-cancer hazard quotient for dermal adsorption (HQ_{der}) is also calculated by dividing the potential dermal exposure by the RfD_O, since no dermal reference dose has been established for these compounds, but taking into account the fraction of contaminant absorbed in gastrointestinal tract (GIABS) as shown in Eq. 2.16. Regarding the inhalation of soil particles (HQ_{inh}), it is calculated by dividing by the RfC, but in this work it was not possible to calculate due to the lack of available data of the RfC of compounds studied (Eq. 2.17). The Hazard Index (HI) is then obtained by summing the individual HQs calculated for the three exposure routes (Eq. 2.18).

Eq. 2.15

$$HQ_{ing} = \frac{Exp_{ing}}{RfD_o}$$

Eq. 2.16

$$HQ_{der} = \frac{Exp_{der}}{RfD_o \times GIABS}$$

Eq. 2.17

$$HQ_{inh} = \frac{Exp_{in}}{RfC}$$

Eq. 2.18

$$HI = HQ_{ing} + HQ_{der} + HQ_{inh}$$

Table 2.2 - USEPA default calculation parameters used in health risk assessment (USEPA, 1991,2011b). Some default values from other institutions are also presented.

Parameter		units	Residential	Occupational	Recreational
Bw	Body weight	kg	70a; 10c[1],15c	70	70a; 10c[1],15c
AT	Averaging time	d	8760NC,a; 2190NC,c 25550C;M	25550C,M	25550C;M
EF	Exposure frequency	d y^{-1}	350	225	240, 26
ED	Exposure duration	y	24a; 6c	25	24a; 6c
ET	Exposure time	hh^{-1}	24/24	8/24	2/24[1]
IRS	Soil ingestion rate	mg d^{-1}	50a[2],100a; 100c[2],200c	50 [3],100	100a; 200c,500c[1]
IFSad	Soil ingestion factor adjusted	res: mg y $kg^{-1}d^{-1}$ rec: mg kg^{-1}	114C; 489.5M	-	2971C; 12728M
SA	Surface area	cm^2 d^{-1}	5700a; 2800c	3300	5700a; 2800c
DSFad	Soil dermal contact factor adjusted	res:mgy$kg^{-1}d^{-1}$ rec: mg kg^{-1}	361C; 1445M	-	9381C; 37582M
AF	Adherence factor soil to skin	mg cm^2	0.07a; 0.2c	0.2	0.07a; 0.2c
PEF	Particle emission factor	m^3 kg^{-1}	$1.4x10^9$	$1.4x10^9$	$1.4x10^9$
10^6	Correction factor	mg kg^{-1}	-	-	-

a – adult; c - children; NC – non-cancer; C – cancer; M - mutagenic

[1]Germany; [2]Dutch; [3]Spain (values taken form Carlon, 2007)

The methodology proposed by the Dutch guidelines has a similar approach, however it is simpler. The exposure from all pathways is calculated for children (6 yrs) and adults (64 yrs) separately (example of ingestion route is shown in Eq. 2.19) and then integrated (Eq. 2.20). In this case the HQ is calculated by comparing the Exp with the TDI (Table 2.2).

Eq. 2.19

$$Exp_{ing} = \frac{C_{soil} \times IRS \times GIABS}{Bw \times 10^6} \ (mg\ kg^{-1}\ d^{-1})$$

Eq. 2.20

$$Exp_{ing}(\text{lifelong}) = \frac{\left(6 \times Exp_{ing}\text{Children}\right) + \left(64 \times Exp_{ing}\text{Adult}\right)}{70} \ (mg\ kg^{-1}\ d^{-1})$$

Table 2.3 - Compound specific parameters used in health risk assessment.

	RfD_O[a]	TDI[b]	CEV[b]	CSF_O[a]	IUR[a]	GIABS[a]	ABS[a]
	Chronic oral reference dose	Tolarable daily intake	Critical exposure value 10^{-4} TLCR	Cancer slope factor	Inhalation unit risk	Fraction of contaminant absorbed in gastrointestinal tract	Dermal absorption from soil
	mg kg^{-1} d^{-1}	mg kg^{-1} d^{-1}	mg kg^{-1} d^{-1}	(mg kg^{-1} d^{-1})$^{-1}$	(mg m^{-3})$^{-1}$	-	-
NP	$2x10^{-}2$	$4x10^{-2}$	-	-	-	-	-
ACY	-	-	$5x10^{-2}$	-	-	-	-
ACE	$6x10^{-2}$	-	$5x10^{-1}$	-	-	-	-
FLU	$4x10^{-2}$	$4x10^{-2}$	-	-	-	-	-
PHE	-	$4x10^{-2}$	-	-	-	-	-
ANT	$3x10^{-1}$	$4x10^{-2}$	-	-	-	-	-
FLA	$4x10^{-2}$	-	$5x10^{-2}$	-	-	-	-
PYR	$3x10^{-2}$	-	$5x10^{-1}$	-	-	-	-
BAA	-	-	$5x10^{-3}$	-	-	-	-
CRY	-	-	$5x10^{-2}$	-	-	-	-
BBF	-	-	$5x10^{-3}$	-	-	-	-
BKF	-	-	$5x10^{-3}$	-	-	-	-
IND	-	-	$5x10^{-3}$	-	-	-	-
DBAH	-	-	$5x10^{-4}$	-	-	-	-
BGHI	-	$3x10^{-2}$	-	-	-	-	-
BaP(eq)	-	-	$5x10^{-4}$	7.3	1.1	1	0.13
PCBs	$5.3x10^{-6}$	$1x10^{-5}$	-	2	0.57	1	0.14

[a]USEPA, 2011b, IRIS (http://www.epa.gov/iris/); [b]Swartjes et al., 2012; Verbruggen, 2012

2.5.2 Cancer risk

The cancer risk is defined by USEPA as "*the incremental probability of an individual's developing cancer over a lifetime as a result of exposure to a potential carcinogen*" (USEPA, 1991). The estimation of cancer risk associated with exposure to a carcinogenic or potentially carcinogenic substance is based on chronic oral slope factors and inhalation unit risks (Eq. 2.21). They are used to estimate an upper-bound probability of an individual's developing cancer as a result of a lifetime of exposure to a certain level of a carcinogen (USEPA, 1991).

Eq. 2.21

$$Cancer\ Risk = Exposure \times Slope\ Factor$$

A slope factor is a plausible upper bound estimate of the probability, approximating a 95% confidence limit, of a response per unit intake of a contaminant over a lifetime. It is usually expressed as the proportion (of a population) affected per mg of substance/kg body weight-day. A similar term, known as inhalation unit risk (IUR), is used to assess inhalation risks, where the exposure-response relationship refers to concentrations in the air. The IRIS[1] database uses a cancer weight-of-evidence descriptor to describe a substance's potential to cause cancer in humans and the conditions under which the carcinogenic effects may be expressed. According to this classification, only 7 of the PAHs (BAA, CRY, BBF, BKF, BAP, IND, DBAH) are considered possible carcinogenic in humans (Table 3 of Annex I). However, since only BAP has a chronic oral slope factor (CSF_O) attributed (Table 2.3), concentrations of other compounds need to be converted to a BAP equivalent concentration by using toxic equivalent factors (TEFs). Since other institutions have other classifications for the carcinogenicity of PAHs (Table 3 of Annex I), and for example RIVM calculated the critical exposure values (CEV) based on a cancer risk also for ACE, ACY, FLA and PYR, it was decided to consider all compounds in the calculation of BAPeq using TEF values given by Tsai et al. (2004) and presented in Annex I (Table 3). Therefore, TEFs were used to calculate the toxic equivalents and to quantify the PAH toxic potency of soil samples, expressed as BAP-equivalents (BAPeq). In the case of PCBs, which can also be considered probable human carcinogen, it is attributed a slope factor for the co-planar PCBs however, in this study only one of these compounds was monitored (PCB 118) (USEPA, 2011b). Considering this it was decided to use the cancer slope factor given by IRIS for the ∑PCBs, considering the highest risk (Table 2.3), which is the one advised for the route of exposure considered and due to the compositional pattern of the mixture (predominance of congeners with more than 4 chlorines).

The potential exposure to the amount of HOCs contained in soil was separately calculated for each route (ingestion, dermal absorption and inhalation of particulates), as described for non-cancer risks. The USEPA procedure presents also models to calculate mutagenic risks associated to the exposure of contaminants and since PAHs can have mutagenic effects, these risks were also calculated. Therefore, cancer and mutagenic lifetime risks for ingestion (LR_{ing}) and dermal adsorption (LR_{der}) were calculated by multiplying the predicted oral or dermal exposure by the CSF_O, since no dermal cancer slope factor has been established for these compounds. Regarding the inhalation of soil particles, risks (LR_{inh}) were calculated by multiplying the exposure by the predicted IUR. Three types of land uses were considered for carcinogenic risk (residential, occupational, and recreational) and two for mutagenic risks (residential and recreational) (USEPA,

[1] http://www.epa.gov/iris/

2011b). The equations Eq. 2.22 and Eq. 2.23 were used for calculating carcinogenic risks in residential soils for ingestion exposure route, whereas Eq. 2.24 and Eq. 2.25 were used for the dermal contact route and Eq. 2.26 was used for the inhalation route. The definition of the variables used in theses equations can be consulted in Table 2.2 and Table 2.3. Equations used to calculate carcinogenic risks for occupational and recreational land as well as mutagenic risks for residential and recreational land use are presented in Annex I.

Eq. 2.22

$$LR_{ing} = \frac{C_{soil} \times EF \times IFS_{ad}}{AT \times 10^6} \times CSF_o$$

Eq. 2.23

$$IFS_{ad} = \frac{ED_c \times IRS_c}{BW_C} + \frac{ED_a \times IRS_a}{BW_a} (mg\ y\ kg^{-1}d^{-1})$$

Eq. 2.24

$$LR_{der} = \frac{C_{soil} \times EF \times DSF_{ad} \times ABS}{AT \times 10^6} \times \frac{CSF_o}{GIABS}$$

Eq. 2.25

$$DSF_{ad} = \frac{ED_c \times SA_c \times AF_c}{BW_C} + \frac{ED_a \times SA_a \times AF_a}{BW_a} (mg\ y\ kg^{-1}d^{-1})$$

Eq. 2.26

$$LR_{inh} = \frac{C_{soil} \times EF \times ED_{a+c} \times ET}{AT \times PEF} \times IUR$$

All exposure parameters, including the age-dependent adjustment factor for mutagenic and carcinogenic risks were first selected according to the EPA's default values (Table 2.2 and Table 2.3). An exception was made for recreational land use, where it was considered an exposure frequency of 26 days and an exposure time of 2 hours.

The acceptable risk for non-threshold compounds is expressed in terms of excess total lifetime cancer risk (TLCR) and varies between 10^{-4} and 10^{-6}; meaning that the probability of an individual develops cancer over lifetime is between one in 10,000 and one in 1,000000. The TLCR for each pollutant was obtained by adding the individual cancer risks calculated in each pathway of concern (i.e., inhalation, ingestion, and dermal absorption), as shown in Eq. 2.27.

Eq. 2.27

$$TLCR = LR_{ing} + LR_{der} + LR_{inh}$$

The methodology proposed by the Dutch guidelines is similar to the one described for non-threshold compounds. The exposure from all pathways is calculated for children (6 yrs) and adults (64 yrs) separately (example of ingestion route is shown in Eq. 2.19) and then integrated (Eq. 2.20). In this case the HQ is calculated by comparing the Exp with the CEV (Table 2.2).

2.6 REVIEW OF RELEVANT GUIDELINES OF SELECTED HOCS IN SOILS

Many countries have developed their own legislation regarding soil protection. A review of existing guidelines worldwide, for non-carcinogenic and carcinogenic PAHs, was made by Jennings (Jennings, 2012a,2012b). From these reviews is possible to observe the variability of existing guidelines. For example, just in eighth autonomous Native American jurisdictions there are 96 regulatory guidance values for the seven carcinogenic PAHs. Guidelines within European countries are also widely variable, as result of differences in the objectives set and derivation methods, and because in some cases they were developed for different regulatory applications (Carlon, 2007; Jennings, 2012a,2012b; Provoost et al., 2006). One of the consequences of such a wide variety of guidance values is the different denomination given by the countries (e.g. screening, target, trigger, cut off or intervention values), which can be related to the objectives set (Carlon, 2007). The guidelines may differ on the target receptor since they can be derived in order to protect human health, ground water quality or biota, following risk assessment models as described in the previous sections. Another source of variability may be because some of the existing guidelines discriminate the land use and soil derived risks differ according to its use. In other cases, values may depend of the type of soil. Moreover, guideline values may be given for the total group of HOCs (typically ∑16PAHs or ∑7PCBs defined in section 2.3), whereas in other cases values are given for individual compounds. The present study will be focused on some selected guidelines, which seems to be the most relevant and complete: Canada, United States, Netherlands, Denmark, Germany, Finland, Italy and Spain.

In general, the criteria have three different levels related to the potential risks (Carlon, 2007; Fernández et al., 2006): screening or target, warning or trigger and intervention or cut off values. The screening or target values refer to concentrations below which there is a negligible risk, and are used to define long term environmental objectives. The aim is to avoid that any type of adverse effect occur even in the most sensitive land use, and it can be obtained based on the natural average background values (Carlon, 2007). Above the warning or trigger values the potential risks are considered intermediate and there is a need of further investigation. The intervention values refer to severely contaminated sites, where the risks are unacceptable and

that need immediate clean-up. Most of guidelines indicate the need of a site-specific RA for areas in which concentrations are above the intervention or cut-off values, whereas in other cases this site-specific RA is advised also in cases where levels are above the target or warning values. The guideline values may be derived separately for human health or ecosystem protection and then then integrated in one value by choosing the lowest between the two.

The generic Dutch guidelines, which were the first to be developed in Europe, refer two values: the target and the intervention value (Table 2.4). The former were achieved after an extensive survey of undisturbed soils and are long term environmental objectives, whereas the last are based on both human health and ecotoxicological risks (the lower value is considered) and define an urgency of remediation (Swartjes et al., 2012). In this particular case of the Dutch guidelines the recommendations are: values below target levels are considered clean and no further action is needed; values between background and intervention values are considered slightly contaminated and a sustainable soil management is needed; and finally, soils with concentrations above the intervention values are seriously contaminated and they need urgent remediation (Swartjes et al., 2012). In order to implement a sustainable soil management the Dutch government introduced the "National Maximal Values", which were derived for relatively immobile contaminants (e.g. PAHs and PCBs), and are "based on risks for human health, the ecosystem and agricultural production" (Swartjes et al., 2012). These values are related to the land use, between the target value (TV) and the intervention value (IV), and allow a new classification in three categories: always suitable (<TV), suitable for residential (<$MV_{residential}$), suitable for industrial (<$MV_{industrial}$) and not suitable (>IV).

Table 2.4 - Generic Dutch guidelines for standard soil (10% organic matter; µg kg^{-1}) (Swartjes et al., 2012).

Compound	Target value	Maximal Value Residential	Maximal Value Industrial	Intervention Value
∑10PAHs[b]	1,000[a]/1,500	6,800	40,000	40,000
∑7PCBs	20	20	500	1,000

[a]Former value presented in the legislation and widely used (VROM, 2000); [b]NP, PHE, ANT, FLA, BAA, CRY, BKF, BAP, IND, and BGHI.

Another example of generic guidelines are the ones from Germany (Table 2.5), which are defined as precaution levels, i.e., values above which there is a certain chance of future soil problems which needs to be addressed in order to avert upcoming damages. These guidelines are

dependent of the organic matter (OM) content. Threshold values from Finland (Table 2.5) can be based on the upper estimate of background concentrations (e.g. PAHs) or on risk limits considering both human health and ecological risks (e.g. PCBs). These threshold values are used as a trigger values, and a site-specific RA is conducted if levels are exceeded. The Finish legislation also presents a lower and upper guideline values, which corresponds to unacceptable risks, and are based on both ecological and human health.

Table 2.5 - Precaution levels from Germany and threshold values from Finland (μg kg^{-1}) (Carlon, 2007).

Compound	Germany	Finland[d]
Naphthalene	-	1,000
Phenanthrene	-	1,000
Anthracene	-	1,000
Fluoranthene	-	1,000
Benzo(a)anthracene	-	1,000
Benzo(k)fluoranthene	-	1,000
Benzo(a)pyrene	300[a]; 1,000[b]	200
∑16PAHs	3,000[a]; 10,000[b]	15,000
∑7PCBs	50[a; c]; 100[b; c]	1,000

[a]≤8%OM; [b]>8%OM; [c]∑6PCBs; [d]standard soil with 10% of OM

2.6.1 Environmental health

A soil quality criterion for ecological receptors is limited when compared to aquatic or human receptors, being the protection of human health the basis of most of guidelines. Many Member States have or are developing ecologically based threshold soil concentrations, although these have yet to be fully integrated into soil quality standards (Carlon, 2007). In general, the guidelines described below were derived using the procedures from the European Commission Technical Guidance Document on Risk Assessment (EC, 2003), as described in section 2.4. Usually, the lower values are derived from ecotoxicological data of the most sensitive species combined with an assessment factor, or from the species sensitive distribution (SSDs) curves (normally the HC_5 is considered). The highest values are normally based on the geometric mean of ecotoxicological data of several species or based on the SSDs and in this case the HC_{50} is normally considered. Even so, values can be very different depending on the country, not only due to minor differences in the scientific methodology but also due to the endpoint of protection or to the land use considered, or even due to political reasons.

The Dutch derived environmental risk limits for direct terrestrial ecotoxicity, specifically for PAHs (Verbruggen, 2012): the maximum permissible concentration (MPC) and the serious risk concentration (SRC). These values were already presented in section 2.4, as well as the methodologies used to derive them, yet they will be again referred for comparison purposes. The MPC values are concentrations that should protect all species in the ecosystem from adverse effects (Table 2.6). Based on these levels (by dividing MPC by 100) it were derived the negligible concentrations (NC), which are levels causing negligible effects to ecosystems and are related to the target values. The SRC (Table 2.6), which are concentrations at which soil functions or species will be seriously affected, can be set as the Intervention Value (after comparing with values derived for human health protection).

Table 2.6 - Guidelines from different European countries for protection of environmental health (values in µg kg^{-1}) (Carlon, 2007; Verbruggen, 2012).

Compound	Netherlands[a]			Finland[a]		Denmark
	NC	MPC	SRC	lower value	upper value	
NP	6.9	690	14,000	5,000	15,000	-
ACY	1.7	170	9,400	-	-	-
ACE	6.8	680	31,000	-	-	-
FLU	16	1,600	82,000	-	-	-
PHE	36	3,600	90,000	5,000	15,000	-
ANT	3.4	340	60,000	5,000	15,000	-
FLA	48	4,800	309,000	5,000	15,000	-
PYR	18	1,800	53,000		-	-
BAA	1.9	190	91,000	5,000	15,000	-
CRY	16	1,600	38,000	-	-	-
BBF	7.9	790	62,000	-	-	-
BKF	7.9	790	44,000	5,000	15,000	-
BAP	1.6	160	76,000	-	15,000	100
IND	3.8	380	89,000	-	-	-
DBAH	1.8	180	18,000		-	-
BGHI	4.9	490	10,000	-	-	-
∑16PAHs	-	-	-	30,000	100,000	1,000[c]
∑7PCBs	-	-	-	-	5,000	10

[a]for a standard soil with 10% OM; [c]∑5PAHs (FLA, (BBF+BJF+BKF), BAP, IND and DBAH).

The Finish guidelines establish a lower and an upper guideline value based on significant risks to soil ecosystem, which are applicable to residential and industrial areas respectively. The lower guideline value is derived from the SSDs (considering the HC_{50}) and the upper guideline value is based on the upper confidence limit (95%) of the median in the SSD. However, when there are no

SSDs data, the lower guideline value may be derived from the geometric mean of ecotoxicological endpoints (e.g. NOEC) and then applying an assessment factor, and in this case the upper guideline value is set as two times the median value.

The Danish EPA establishes ecotoxicological soil quality criteria (Table 2.6) based on NOEC, ECx or LOEC for soil dwelling organisms, plants, microorganisms and microbiological processes. The aim is to protect the function and the structure of the ecosystems. Values were derived only for BAP, for the sum of 5 PAHs and for the sum of PCBs (Table 2.6) but they are very conservative when compared with other proposed guidelines.

The Spanish guidelines (Table 2.7) refer to generic reference values for the protection of ecosystems for individual compounds which were derived for a natural soil use (MP, 2005). The values were calculated based on the ratio between the predicted environmental concentration and the PNEC values for different environmental compartments (water column, soil and wildlife food). These are considered trigger values, meaning that if exceeded a site-specific risk assessment should be conducted.

Table 2.7 - Spanish guidelines for protection of environmental health (values in μg kg^{-1}, standard soil with 3.4% OM).

Compound	Soil organisms	Aquatic organisms	Terrestrial vertebrates
NP	100	50	60
ACE	-	20	4,850
FLU	220	20	2,840
ANT	-	10	22,000
FLA	1,000	30	1,960
PYR	-	10	1,200
BaA	3,800	10	-
BaP	150	10	-

The Canadian Council of Ministers of the Environment (CCME) established ecological risk-based numerical standards aimed to protect the key ecological receptors that sustain normal activities within four defined land use categories: agricultural, residential/parkland, commercial or industrial (CCME, 2008). The soil quality guidelines for environmental health protection (SQG_E) are based on non-carcinogenic effects of PAHs and take into account the levels of 15 PAHs (Table 2.8).

These SQG_E values represent the lowest of the available environmental health guidelines (soil contact, soil and food ingestion, protection of freshwater life) (CCME, 2010). When there is insufficient data to calculate SQG_E or provisional SQG_E, an interim soil quality criterion is given.

These SGQ are intended to "ensure that no adverse effects of biological importance are likely to occur, based on toxicological data associated with acute, subchronic and chronic responses in representative species" (CCME, 2008). Values in bold presented in Table 2.8 are the ones that should be used in first screening of generic environmental risks.

Table 2.8 - CCME guidelines for environmental health protection (values in μg kg^{-1}).

Compound	Agricultural	Residential/Parkland	Commercial	Industrial
NP	**600**[a]; 13[b];8,800[c]	**600**[a]; 13[b]; 8,800[c]	**22,000**[a]; 13[b]	**22,000**[a]; 13[b]
ACY	320,000[b]	320,000[b]	320,000[b]	320,000[b]
ACE	280[b];21,500[c]	280[b];21,500[c]	280[b]	280[b]
FLU	250[b];15400[c]	250[b];15,400[c]	250[b]	250[b]
PHE	46[b];43,000[c]; **100**[d]	46[b];43,000[c]; **5,000**[d]	46[b]; **50,000**[d]	46[b]; **50,000**[d]
ANT	**2,500**[a;e];61,500[c]	**2,500**[a;e];61500[c]	32,000[a;e]	32,000[a;e]
FLA	**50,000**[a;e];15,400[c]	**50,000**[a;e];15,400[c]	180,000[a;e]	180,000[a;e]
PYR	7,700[c];**100**[d]	7,700[c];**10000**[d]	100,000[d]	100,000[d]
BaA	6,200[c]; **100**[d]	6,200[c]; **1,000**[d]	10,000[d]	10,000[d]
CRY	6,200[c]	6,200[c]	-	-
BbF	6,200[c]; **100**[d]	6,200[c]; **1,000**[d]	10,000[d]	10,000[d]
BkF	6,200[c]; **1000**[d]	6,200[c]; **1,000**[d]	10,000[d]	10,000[d]
B(b+j+k)F	100[d]	1,000[d]	10,000[d]	10,000[d]
BaP	**20,000**[a;e];600[c];8800,000[b]	**20,000**[a;e];600[c];880,0000[b]	**72,000**[a;e];8800,000[b]	**72,000**[a;e];8800,000[b]
IND	100[d]	1000[d]	10,000[d]	10,000[d]
DahA	100[d]	1000[d]	10,000[d]	10,000[d]

[a]SQG for environmental health; [b]SQG for protection of freshwater life; [c]SQG for environmental health through soil and food ingestion [d]Interim SQC; [e]SQG for environmental health through soil contact

2.6.2 Human Health

According to the Canadian guidelines, the goal of the human health risk-based numerical standards is "*to ensure that exposure via the identified land use-specific exposure pathways would not lead to an overall level of human exposure in excess of that considered tolerable from a toxicological standpoint*" (CCME, 2008).

Normally, guidelines derived for non-carcinogenic compounds are based on a hazard quotient (HQ) of 1 (Eq. 2.11). However, for carcinogenic compounds the target total lifetime cancer risk (TLCR, as shown in Eq. 2.27) can be different, normally ranging from 1 in 10,000 (10^{-4}) to 1 in 1000,000 (10^{-6}). Other differences can be due to land use scenarios considered, correction or not for soil type, pathways and conditions of exposure, inclusion or not of non-soil sources of exposure or even due to toxicological data used (Carlon, 2007).

The human health-based risk limits from the Dutch guidelines (Table 2.9) are based on the tolerable daily intake (TDI) and on the critical exposure value (CEV), which in the case of PAHs are

shown in Table 2.3. Risks are derived for an residential exposure scenario using the model for oral ingestion (not only soil ingestion but also from other routes such as consumption of vegetables) presented in Eq. 2.19 and Eq. 2.20, but the inhalation pathway (e.g. volatilization from soil or water, soil particles) is also considered (Swartjes et al., 2012). The intervention values (IV) are defined as the concentration of the contaminant in soil for which the sum of HQ (oral+inhalation) is equal to 1 for threshold contaminants and a risk level of 10^{-4} for non-threshold. In addition to the IV, the Dutch guidelines also derived the maximal values for a specific land use scenario (values for residential land use are shown in Table 2.4) and in this particular case for threshold contaminants the TDI is corrected for background exposure and for non-threshold it is set a risk level of 10^{-6}.

The Finish guidelines consider the lowest value obtained when comparing ecological and human health risks. As explained before for the ecological guidelines it is set a lower and an upper guideline value, which are applicable to residential and industrial areas respectively (Carlon, 2007). Only for BAP and ∑7PCBs the lowest values obtained were the ones related to human health risks and correspond to the lower guideline value (Table 2.9). These values consider health risks for both children and adults and several pathways are included (ingestion, inhalation and dermal contact, and consumption of home-grown vegetables). The risk limit set for non-carcinogenic is 1 whereas for carcinogenic compounds is 10^{-5}.

Table 2.9 - Guidelines for protection of human health (values in μg kg^{-1}) from different countries (Carlon, 2007; CCME, 2010; DEPA, 2002).

Compound	Netherlands[a;b]	Finland[a;c;d]	Denmark[c]	Canada[h]
NP	870	-	-	-
PHE	23,000	-	-	-
ANT	25,500	-	-	-
FLA	30,300	-	-	-
BAA	3,000	-	-	-
CRY	32,000	-	-	-
BKF	3,200	-	-	-
BaP	280	2000	100[e]; 1000[f]	600[i]; 5,300[c]
IND	3,200	-	-	-
DbaH	-	-	100[e]; 1000[f]	-
BGHI	19,200	-	-	-
∑PAHs	-	-	1500[e;g];15000[f;g]	-
∑7PCBs	-	500	-	-

[a]standard soil with 10% OM; [b]Based on TLCR of 10^{-4}; [c]Based on TLCR of 10^{-5}; [d]Lower guideline value; [e]soil quality criteria; [f]Cut-off criteria; [g]∑5PAHs; [h]BaPeq; [i]Based on TLCR of 10^{-6}.

Table 2.9 also shows the values of Danish guidelines for protection of human health, based on toxicological quality criteria (DEPA, 2002). Two values are given: soil quality criteria, based on chronic effects and acceptable/tolerable daily exposures, and cut-off criteria based on chronic harmful effects. When the quality criteria are exceeded exposure should be reduced and this levels are indicated for sites with very sensitive uses (e.g. private gardens or day-care centres). The cut-off values indicates the level above which remediation is needed.

In the particular case of Canadian guidelines for human health protection, it is intended to ensure that humans are protected from direct contact with contaminated soil. The guidelines are based only in the effects of carcinogenic PAHs (BAA, BAP, BBF, BKF, BJF, CRY, DBAH, BGHI, IND) and two values are given corresponding to the risk limit set: a TLCR of 10^{-5} or 10^{-6} (Table 2.9). Yet, it should be noted that values are given as (BAPeq), which are calculated as described in section 2.5.2. The recommended values are the same for the different land uses (agricultural residential/parkland, commercial or industrial).

The German guidelines recommend different values according to the land use. The values presented in Table 2.10 are trigger values, i.e, above these values further investigations should be conducted in order to verify if these values represent or not a hazard. Levels are based on a TLCR of 10^{-5} (threshold contaminants) and two pathways are considered (soil ingestion and the inhalation of dust particles), but the exposition to hazard substances coming from other media such as water or food is also taken into account.

Table 2.10 - Trigger values (µg kg^{-1}) for the soil-human direct contact of German guidelines (Carlon, 2007).

Compound	Playground	Residential	Park/recreation	Industrial
BaP	2,000	4,000	10,000	12,000
∑6PCBs	400	800	2,000	40,000

The Spanish values (Table 2.11) correspond to generic reference values which were derived for a HQ of 1 (for non-carcinogenic) and a TLRC of 10^{-5} (for carcinogenic). The routes of exposure considered depend on the land use and for example for a residential land use it is considered the ingestion, inhalation (soil particles and vapours), and the dermal contact (MMA, 2005; MP, 2005). The proportion of the exposure allocated to the contaminated soil was also taken into account when deriving these guidelines. When values are above the reference guidelines a site-specific

risk assessment should be conducted and when that is not possible it should be considered that there is an unacceptable risk if levels exceed 100 times the reference values.

Another example of legislation that specifies the land use is the Italian (Table 2.11). The methodology was based on the Dutch one, but only human health is considered. A threshold value was defined and if the concentrations are exceeded the soil is considered polluted and a remedial action should be taken. The worst case scenario was considered for the derivation of guidelines for different land uses: three exposure routes of exposure (inhalation, ingestion and dermal contact) for the longest allowable exposure time. A HQ of 1 (for non-carcinogenic) and a TLRC of 10^{-6} (for carcinogenic) were set.

The USEPA preliminary remediation goals are shown in Table 2.11. The screening levels are calculated based on the target risk of 10^{-6} for cancer and 1 for non-carcinogenic, and results from the combined risk of the three routes of exposure (inhalation, dermal and ingestion). The models behind the derivation of these guidelines are the ones presented in section 2.5.1 and 2.5.2

Table 2.11 – Spanish, Italian and USEPA screening levels regarding human health protection (values in μg kg^{-1}) (Carlon, 2007; MP, 2005; USEPA, 2011b).

Comp.	Spain[a]			Italian[b]		USEPA PRG[b]	
	Others	Residential	Industrial	Residential/ Public park	Commercial/ industrial	Residential	Industrial
NP	1,000	8,000	10,000	5,000	50,000	$1.4X10^5$	$6.2\ X10^5$
ACE	6,000	60,000	100,000	5,000	50,000	$3.4X10^6$	$3.3X10^7$
ACY	-	-	-	5,000	50,000	-	-
FLU	5,000	50,000	100,000	5,000	50,000	$2.3X10^6$	$2.2\ X10^7$
PHE	-	-	-	5,000	50,000	-	-
ANT	45,000	100,000	100,000	5,000	50,000	$1.7X10^7$	$1.7\ X10^8$
FLA	8,000	80,000	100,000	5,000	50,000	$2.3X10^6$	$2.2X10^7$
PYR	6,000	6,0000	100,000	5,000	50,000	$1.7X10^6$	$1.7\ X10^7$
BaA	200	2,000	20,000	500	10,000	150	2,100
CRY	20,000	100,000	100,000	5,000	50,000	15,000	210,000
BbF	200	200	20,000	500	10,000	150	2,100
BkF	2,000	20,000	100,000	500	10,000	1,500	21,000
BjF	-	-	-	500	10,000	380	1,300
BaP	20	200	2,000	100	10,000	15	210
DahA	30	300	3000	100	10,000	15	210
IND	300	3,000	30,000	100	5,000	150	2,100
B(ghi)P	-	-	-	100	10,000	-	
∑PAHs	-	-	-	10,000[c]	100,000[c]		
∑PCBs		-	-	1/60[d]	5,000	740	

[a]Based on TLCR of 10^{-5}; [b]Based on TLCR of 10^{-6}; [c]26 to 34 compounds; [d]proposed in a revision

Chapter 3

THE PREDICTION OF PAHS BIOAVAILABILITY IN SOILS USING CHEMICAL METHODS: STATE OF THE ART AND FUTURE CHALLENGES[1]

[1] Adapted from Cachada A, Pereira R, da Silva EF, Duarte AC. The prediction of PAHs bioavailability in soils using chemical methods: State of the art and future challenges. Sci Total Environ 2014; 472: 463-480.

3.1 INTRODUCTION

The total amount of hydrophobic organic contaminants (HOCs) in soil, given by traditional chemical methods, may not relate directly to environmental or human health risk, since normally only a fraction of contaminants can be leached to groundwater or be uptaken by organisms (Alexander, 2000; Ehlers and Luthy, 2003; Semple et al., 2003). The fraction of contaminants accessible for bioaccumulation (through pore water, soil and food) or transformation by organisms, is normally called available or bioavailable, and is environmentally the most significant (Alexander, 2000; Ehlers and Luthy, 2003; Semple et al., 2003). Therefore, the chemical prediction of bioavailability established by assessing the most soluble or easily extracted fraction has been a major issue in the last years on environmental sciences (Cui et al., 2013; Ehlers and Loibner, 2006).

The major difficulty when dealing with the concept of bioavailability is that there are many definitions and several methods to measure and to calculate it, depending on the specific scientific discipline (Ehlers and Loibner, 2006; Reichenberg and Mayer, 2006; Semple et al., 2004; Semple et al., 2003). Indeed, bioavailability depends on the physical, chemical and biological properties of contaminants, soil and receptors and it is governed by three way interactions between contaminants, matrix and organism (Ehlers and Luthy, 2003; Sijm et al., 2000). Therefore, three distinct processes are involved: physico-chemical, physiological uptake and toxicological. The physico-chemical processes, which have been extensively discussed in recent years (Ehlers and Loibner, 2006; Reid et al., 2000a; Semple et al., 2003), include sorption, diffusion and partitioning and are controlled by soil and compounds properties such as soil organic matter (OM) content and quality, soil inorganic constituents and lipophilicity of compounds. The physiological uptake processes depends on receptor type and specific parameters such as anatomy, feeding strategy or lipid content of organism, whereas toxicological processes are controlled by metabolism, detoxification or accumulation capacity (Ehlers and Luthy, 2003).

Due to the need of incorporate bioavailability in risk assessment, efforts have been made to clarify and standardize the concept and develop methods to measure it (Brand et al., 2013; Ehlers and Luthy, 2003; Reichenberg and Mayer, 2006; Semple et al., 2004). Semple et al. (2004) suggested distinguishing the concepts of bioavailability and bioaccessibility according to their working definitions. The term bioavailability is related to the fraction actually or freely available for the organism at a given time, whereas bioaccessibility is the fraction potentially available over time (it encompasses what is actually bioavailable and what is potentially bioavailable). Further, Reichenberg and Mayer (2006) defined two major processes that rule the physico-chemical

component of bioavailability: chemical activity and bioaccessibility. Chemical activity quantifies the potential of contaminants for spontaneous processes such as partition, sorption or diffusion, being related to fugacity and freely dissolved concentrations. Bioaccessibility is directly comparable with the definition provided by Sample et al. (2004) and refers to the fraction of HOCs that is weakly or reversibly sorbed and can undergo rapid desorption from the solid phase to the aqueous phase. Both processes can be included in a generic concept of chemical availability. In practice, the existing analytical methods to measure chemical availability can be divided in two groups according to the definitions given by Reichenberg and Mayer (Cui et al., 2013). Bioaccessibility is given by depletive non-exhaustive extractions (such as mild solvent or solid phase extraction) and the chemical activity, or freely dissolved concentrations, by non-depletive biomimetic methods (passive sample techniques such as solid phase microextraction or semipermeable membrane devices).

Estimation of bioavailability is traditionally performed by using direct (accumulation in organism's tissues or biodegradation) or indirect (related with effects of contaminants - ecotoxicological tests) bioassays. In fact, bioassays are considered the most accurate approach to assess the available fraction, since they integrate the three way interactions between contaminants, matrix and organism. However, there are some constrains regarding the use of bioassays. For example, regarding field bioaccumulation studies, it is not always possible to measure the contents of contaminants in organism's tissues for all the species (ethical issues, presence of organisms), whereas in bioaccumulation tests the culturing of organisms may be difficult and it can be time consuming since more than one species should be tested. Regarding ecotoxicological tests, the main problem is that generally they are not compound specific. There is a need to develop rapid, accurate, compound specific, cheap, ethical, user- and more environmental- friendly methods, and it has been suggested that non-traditional chemical tests could meet these requirements. Nevertheless, it is necessary to understand and validate the results of chemical methods in order to perform a routine application and its incorporation in risk assessment. Indeed, this is the biggest challenge since bioavailability is species, soil or matrix and compound specific, as is also chemical availability (Kelsey et al., 1997; Reid et al., 2000a; Ten Hulscher et al., 2003). For example, it was already demonstrated that one single chemical method could not predict availability for different organisms and the results depend on the compound as well (Kelsey et al., 1997; Ten Hulscher et al., 2003). In addition to organisms and compound specificity, availability is also matrix specific (e.g.: sorption differences between soils and sediments). For these reasons this review will focus on the existing methods to study chemical

availability of one group of HOCs, polycyclic aromatic hydrocarbons (PAHs) in soils, and the relationship with bioavailability in terrestrial organisms.

Due to the existence of a vast number of diffuse and point sources, their persistence and tendency to accumulate in soils, PAHs can be major contaminants in urban or industrial areas, where levels are often above the recommended guideline values (Cachada et al., 2012a; Cai et al., 2008). Moreover, the importance and interest of studying this group of contaminants is related to their environmental significance (carcinogenic, mutagenic or endocrine disrupting properties), and due to the wide range of properties of the 16 priority PAHs defined by the US EPA (Table 1 of Annex II).

Several chemical methods have been used to predict PAHs bioavailability to bacteria, earthworms and plants (Table 3.1- Table 3.4). Some authors found that chemical methods tested were able to predict bioavailability for a given organism, whereas others stated that it will not be possible (e.g.: Bogan and Sullivan, 2003; Liste and Alexander, 2002). Literature data is scattered and contradictory, and leaves the question on whether it is possible or not to predict PAHs bioavailability using chemical methods unanswered. This study aims at producing a state of the art of what is known about bioavailability and chemical availability of PAHs in soils, clarifying which chemical methods can provide a better prediction of an organism exposure, and which are the most promising ones. The importance of incorporating chemical availability in risk assessment is also discussed.

3.2 REVIEW OF EXISTING CHEMICAL METHODS USED TO PREDICT BIOAVAILABILITY OF PAHS IN SOILS

Chemical methods typically include vigorous extractions, normally called total or exhaustive, performed by hot solvent (Soxhlet), ultrasonic or accelerated solvent extraction. However, these procedures are not related with the available fraction, since it has been observed that they over predict availability to organisms by a factor that can reach 10-10,000 times and the correlations with bioassay data is normally poor (Gomez-Eyles et al., 2010; Gomez-Eyles et al., 2012; Kelsey et al., 1997). Therefore, other analytical methods that are environmentally more relevant have been developed. Despite measuring different components of the matrix, the non-exhaustive extractions and biomimetic methods, which are the two main approaches used, are based in the principle that the exposure of contaminants to soils organisms occurs mainly through the aqueous phase. Cui et al (2013) recently published a review of the principles, advantages and limitations of

Table 3.1 - Compilation of studies comparing biodegradation and chemical methods.

Chemical Method	Compound	Contamination source	Species	Reference
BuOH	NP	Spiked	*Pseudomonas putida* G7	Kelsey and Alexander, 1997
BuOH	PHE; PYR	Spiked	*M. austroafricanum*	Bogan and Sullivan, 2003
BuOH	PHE, PYR	Spiked	*Pseudomonas putida* G7	Liste and Alexander, 2002
BuOH; 50% MeOH or EtOH	PHE	Spiked	*Pseudomonas R*	Kelsey et al., 1997
Sequential extraction	PYR	Spiked	PYR-degrading (PAS1)	Macleod and Semple, 2003
SWE	PHE	Spiked	*Pseudomonas sp.*	Latawiec et al., 2008
SFE	13 PAHs	Gas, wood and tar works	Indigenous (composting)	Cajthaml and Šašek, 2005
Sequencial SFE	8 PAHs	MGP/railroad, steel, coal tar	PAH-degrading	Szolar et al., 2004
Tenax	PHE	Spiked	*Pseudomonas* R	Braida et al., 2004
Tenax	15 PAHs	MGP	PAH-degrading	Li et al., 2005
HPCD	PHE	Spiked	*Pseudomonas sp.*	Hickman and Reid, 2005
HPCD	PHE	Spiked	Indigenous	Papadopoulos et al., 2007b
HPCD	PHE	Spiked	*Pseudomonas sp.*	Rhodes et al., 2008a; Rhodes et al., 2008b
HPCD	PHE	Spiked	*Pseudomonas sp.*	Rhodes et al., 2010
HPCD	PHE	Spiked	*Pseudomonas sp.*	Allan et al., 2006
HPCD	PHE	Spiked	*Pseudomonas sp.*	Doick et al., 2006
HPCD	4 PAHs	Roadside	Indigenous	Johnsen et al., 2006
HPCD	9 PAHs	Creosote	Indigenous	Sabaté et al., 2006
HPCD	14 PAHs	MGP	Indigenous	Papadopoulos et al., 2007a
HPCD	16 PAHs	MGP/diesel, lubricating oil	PAH-degrading	Hickman et al., 2008
HPCD	16/20 PAHs	Spiked/coke works	*Pseudomonas sp.*	Stokes et al., 2005
HPCD	24 PAHs	Spiked/coke plant	Indigenous/ PAH-degrading	Doick et al., 2005
Persulfate	16 PAHs	MGP/wood works/railroad	PAH-degrading	Cuypers et al., 2000
BuOH; 50% or 100% PrOH; HPCD; Persulfate	12 PAHs	Creosote	Indigenous	Juhasz et al., 2005
BuOH; HPCD	PHE	Spiked	*Pseudomonas sp.*	Reid et al., 2000b
BuOH; SWE; HPCD; surfactants	10 PAHs	Spiked/tar works	*Pseudomonas sp.*	Latawiec and Reid, 2009
BuOH; MeOH; 50% EtOH; surfactants	15 PAHs	Gaswoks/coking plant	Indigenous	Thiele-Bruhn and Brümmer, 2004
SFE; XAD2	20 PAHs	MGP	Indigenous	Hawthorne and Grabanski, 2000; Hawthorne et al., 2001
XAD4; HPCD	NP	Spiked	Indigenous	Patterson et al., 2004
Tenax; HPCD	12 PAHs	Historical sites	Indigenous	Bernhardt et al., 2013
SPME	PHE	Spiked	*Mycobacterium vanbaalenii* (PYR-1)	Yang et al., 2009

Table 3.2 - Compilation of studies comparing earthworm accumulation and chemical methods.

Chemical Method	Compound	Contamination source	Species	Reference
BuOH	PYR; BAA	Spiked	*Aporrectodea longa*	Johnson et al., 2002
BuOH	PYR, CRY	Spiked	*Eisenia fetida*	Liste and Alexander, 2002
BuOH; 50% MeOH; 35% EtOH	PHE	Spiked	*Eisenia fetida*	Kelsey et al., 1997
BuOH; MeOH; PrOH	ANT, FLA, PYR	Spiked	*Eisenia fetida*	Tang and Alexander, 1999
BuOH; SFE	PYR	Spiked	*Eisenia fetida*	Sun and Li, 2005
SFE	PHE; PYR	Spiked	*Eisenia fetida*	Bielská et al., 2013
SFE	16 PAHs	MGP	*Aporrectodea caliginosa*	Kreitinger et al., 2007
XAD4	12 PAHs	MGP	*Eisenia fetida*	Bogan et al., 2005
Tenax	PYR	Spiked	*Eisenia fetida*	Li et al., 2007
Tenax	12 PAHs (+chlorobenzenes)	Field contaminated Soil/sediment	*Lumbricus rubellus*	Ten Hulscher et al., 2003
HPCD	PHE	Spiked	*Lumbricus rubellus*	Hickman and Reid, 2005
BuOH; HPCD	PYR	Spiked	*Eisenia fetida*	Khan et al., 2011
BuOH; HPCD; Tenax	5 PAHs	Spiked	*Eisenia fetida*	Gomez-Eyles et al., 2010
95% EtOH; C18	ANT, PYR, CRY, BAP	Spiked	*Eisenia fetida*	Tang et al., 2002
SPME	13 PAHs	MGP	*Eisenia fetida*	Jonker et al., 2007
C18	15 PAHs	Urban	*Lumbricus terrestris*	Krauss and Wilcke, 2001
TECAM	NP, PHE, PYR, BAP	Spiked	*Eisenia andrei*	Tao et al., 2008b
TECAM	NP, PHE, PYR, BAP	Field contaminated	*Eisenia andrei*	Tao et al., 2009
BuOH; HPCD; SPME; POM-SPE	12 PAHs	Industrial	*Eisenia fetida*	Gomez-Eyles et al., 2012
50% MeOH; 1% MeOH or BuOH; Sequential leaching; surfactant; HPCD; SPME; SPMDs	15 PAHs	Gas plant	*Eisenia fetida*	Bergknut et al., 2007

Table 3.3 - Compilation of studies comparing plant accumulation and chemical methods.

Chemical Method	Compound	Contamination source	Species	Reference
BuOH; MeOH; PrOH	ANT	Spiked	Wheat; Barley	Tang and Alexander, 1999
Tenax	16 PAHs	MGP	*Festuca arundinacea; Panicum virgatum*	Cofield et al., 2008
Butanol; HPCD; Tenax	5 PAHs	Spiked	*Lolium multiflorum*	Gomez-Eyles et al., 2010
BuOH; 50% MeOH;TECAM	NP, PHE, PYR, BAP	Field contaminated	*Triticum aestivum L.*	Tao et al., 2008a
BuOH; HPCD; SPME; POM-SPE	12 PAHs	Industrial	*Lolium multiflorum*	Gomez-Eyles et al., 2012

Table 3.4 - Compilation of studies comparing ecotoxicological tests and chemical methods.

Chemical Method	Compound	Matrix	Test	Reference
BuOH	BAP	Spiked	Bacterial genotoxicity	Alexander and Alexander, 2000
SFE	16 PAHs	MGP	Earthworm toxicity	Kreitinger et al., 2007
SFE	13 PAHs	Wood/ gas works	Earthworm and plant growth inhibition and mortality; bioluminescent inhibition	Čvančarová et al., 2013
Tenax	16 PAHs	Gas plant	Nematode and earthworm survival, lettuce emergence, microbial respiration	Cofield et al., 2008
HPCD	PHE	Spiked	^{1}H NRM metabolomics	Brown et al., 2010
SPME	PYR	Spiked	Springtail toxicity	Styrishave et al., 2008
SPME	13 PAHs	MGP	Earthworm toxicity	Jonker et al., 2007

the chemical methods most commonly used to predict the bioavailability of HOCs in soils and sediments. The paper also proposes operational protocols for the methods and guidelines to interpret data obtained, i.e. how to compare chemical availability with bioassay results. Focusing on methods that predict the bioavailability of PAHs in soils, compiling major findings and reviewing the knowledge about chemical availability of these compounds are the specific goals of this work.

3.2.1 Non-exhaustive extractions

Non-exhaustive methods consist in simple shaking extractions to measure the fraction known as bioaccessible. They give the mass quantity of contaminants, which are or can become available under given conditions and a within time period (Reichenberg and Mayer, 2006; Semple et al., 2004). Therefore, measuring the desorption kinetics of contaminants can estimate bioaccessibility. It is believed that non-exhaustive methods may predict the bioavailable fraction because as uptake by organisms occurs, there is a depletion of the freely dissolved concentrations which may be replenished by the weakly or reversibly sorbed fraction.

Because HOCs are not uniformly distributed in particles, desorption kinetics is triphasic, due to the depletion of the contaminant pools from different compartments at rates inversely related to contaminant-particle sorption strength (Rhodes et al., 2010; You et al., 2011). The data is normally fitted to a three-compartment desorption model, assuming first order kinetics for each of the three compartments (Cui et al., 2013; You et al., 2011): $S_t/S_0=F_{rap}(e^{-krap*t})+F_s(e^{-ks*t})+F_{vs}(e^{-kvs*t})$. Where, S_t and S_0 represent the concentration in soil at time t and 0, respectively; F_{rap}, F_s and F_{vs}, represent the rapid, slow and very slow desorbing fractions at time zero and krap, ks and kvs are the respective desorption rate constants. The first phase, F_{rap}, includes the sorbed contaminants which can desorb towards the pore water within a short time, while the remaining phases, F_s and F_{vs}, are considered kinetically rate-limited. The F_{rap} is assumed to be the representative of release conditions and, based on this, the assessment of this fraction has been suggested as a rapid approach to measure bioaccessibility, since the study of desorption kinetics including the F_s and F_{vs} may take several weeks or months. Still, this approach is used as a proxy and may under- or overestimates the F_{rap}. While some methods (solid phase extraction or solubilising agents) have shown to be able to remove only the F_{rap}, under a defined time frame, others, such as mild solvent extraction, may also remove the F_s (Kelsey et al, 1997). Indeed, one of the difficulties in interpreting and comparing results is the fact that bioaccessibility is operationally defined, and

different experimental conditions will give different results (e.g.: type of agitation, soil: solution ratio, desorption solution used or desorption time).

Bioaccessibility tends to decrease with increasing matrix contact time (sequestration), i.e., chemical available fractions (or extractability) declines with residence time of compounds in soil in opposition to fractions obtained by vigorous extractions. This was demonstrated for the methods discussed: mild solvent (Johnson et al., 2002; Kelsey et al., 1997); supercritical fluid extraction (Bogolte et al., 2007; Sun and Li, 2005) solid phase extraction (Li et al., 2007); solubilising agents (Khan et al., 2011; Rhodes et al., 2008b; Swindell and Reid, 2006). Similarly, extractabilities of PAHs are expected to decrease with the increase of soil OM content, while the total content of PAHs usually increases with the OM content (Bogan and Sullivan, 2003; Cachada et al., 2012a). Still, this behaviour was not always verified, suggesting that other factors may also have influence on sequestration and desorption behaviour of PAHs (Hawthorne et al., 2002). It is also expected that non-exhaustive extractions reflect individual PAH properties: in spite of being normally more abundant in naturally contaminated soils, high molecular weight (HMW) PAHs tend to have lower extractabilities due to their recalcitrant nature and higher tendency to associate with OM (Bogan et al., 2005; Hawthorne and Grabanski, 2000).

Mild solvent extraction: this is a very simple approach that involves the agitation of soil with polar solvents (typically primary alcohols), or a mixture of solvent with water, for a determined period of time, and finally the analysis of PAHs in the extraction solvent (Cui et al., 2013). The solvent most commonly used is n-butanol (BuOH), but others such as methanol (MeOH), ethanol (EtOH) or propanol (PrOH) have been also tested (Tang and Alexander, 1999; Tang et al., 2002). In addition to the different solvents used, different operational conditions (Table 2 of Annex II) turned the comparison of results very difficult to perform. Moreover, very few studies tested the 16 PAHs, being the majority focused on 3- and 4-ring compounds.

Some authors used sequential leaching, i.e., solvents of decreasing polarity, to assess the relative sorption strength of various PAHs pools. For example, Bergknut et al (2007) used a sequence of MeOH, BuOH, acetone, n-hexane and toluene to extract PAHs from a gaswork's site. However, these authors found that MeOH already extracted 83% of the ∑15 PAHs. Also, Macleod and Semple (2003) used a sequential extraction with 50% MeOH, BuOH and dichloromethane to extract pyrene (PYR) from spiked soils and, for 24 weeks aging, the percentages extracted ranged between 0.8-2.2% for the first step, 32-46% for the second and 22.5-24.7% for the final step. These results suggest that extractable percentages could be close to exhaustive extractions, when

using pure MeOH or BuOH. Indeed, other studies using single BuOH extractions reported extractabilities of individual compounds in naturally contaminated samples ranging between 41 and 72%, either using a 48h or a 120s vortex extraction (Latawiec and Reid, 2009; Tao et al., 2008a). Moreover, no differences were observed when extracting naphthalene (NP) in freshly spiked and in aged soils (99.9 and 93.3%, respectively) (Kelsey and Alexander, 1997).

In addition, the pattern of individual compounds extracted by MeOH was found to be similar to total extractions (Bergknut et al., 2007). Likewise, BuOH extracted a similar amount of 2 to 5-ring PAHs in 15 naturally contaminated soils: 55.1±3.7% for NP, 44.3±3% for PHE, 53.8±4.3% for PYR and 52.2± 4.7% for benzo(a)pyrene (BAP) (Tao et al., 2008a). In another study it was observed that BuOH extractabilities of individual compounds, in a naturally contaminated soil, increased with the octanol-water partition coefficient (K_{OW}): 41% for NP to 72% for benzo(a)anthracene (BAA) (Latawiec and Reid, 2009). Again, it's noteworthy that different BuOH contact times seem to have a little effect on PAH extraction pattern (Gomez-Eyles et al., 2010). On the other hand, using a mixture with 50% MeOH, it was possible to relate the fraction of individual PAHs extracted with their properties. In urban soils, for instance, the extractability ranged between 68% for acenaphthene (ACE) and 5.9% for benzo(ghi)perylene (BghiP), decreasing linearly with increasing log K_{OW} (Krauss et al., 2000). Tao et al. (2008a) found that in 15 field contaminated soils this mixture extracted 8.8±0.67% for NP, 5.16±0.43% for PHE, 3.66 ±0.27% for PYR and 1.17±0.07 % for BAP. Moreover, decreasing the amount of solvent drastically reduces the percentage of PAHs extracted: 83% using pure MeOH, 5% using 50% MeOH and 0.16 or 0.13% using either 1% MeOH or BuOH for the ∑15 PAHs (Bergknut et al., 2007). Still, the pure BuOH extractability has been related to soil properties: in 13 coal tar soils aged for 120 days, the fraction of PAHs (3 to 6 ring) extracted with this solvent was found to be inversely correlated to the OC content (Bogan and Sullivan, 2003).

Subcritical water extraction (SWE): Latawiec et al (2008) suggested SWE as a promising method to predict long-term release rates of PAHs from soils. The principle is to lower the polarity of water by increasing its temperature (subcritical water when T<374°C) while maintaining the liquid state by controlling the pressure. This method allows changing the water properties to obtain an aqueous solvent with similar properties to organic solvents. These authors performed an extraction similar to the conventional pressurised liquid extraction and tested several conditions, reaching the conclusion that 200 °C during 10 min was the best option (Latawiec and Reid, 2009). However, only these two studies were undertaken, and only in the latest several

compounds were analysed (10 PAHs with 2-,3- and 4-ring) in a field contaminated soil. A decrease in extractability according to the ring size, ranging from 17% for NP to 2% for anthracene (ANT) and BaA was observed. The authors advise that there is a need to understand the influence of factors such as soil ratio, dispersing agent or water volume.

Supercritical fluid extraction (SFE): SFE with pure CO_2 has been suggested as a rapid method to study the available fraction of PAHs in soils. Pure CO_2 has been chosen since it has a polarity similar to biological lipids, and, when using appropriate conditions of temperature and pressure, the solubility of PAHs is similar to the ones of water (Hawthorne et al., 2001). Yet, supercritical CO_2 is considered relatively lipophilic, transferring HOCs from nonpolar matrices more efficiently than water. It easily allows a characterization of the desorption kinetics by performing several extraction phases (sequential leaching) with successively harsher conditions (increase fluid temperature and density) that mobilize compounds from different soil particle sites (Hawthorne and Grabanski, 2000; Hawthorne et al., 2001; Szolar et al., 2004). One of the major advantages is that this method does not alter the soil organic matrix (Hawthorne and Grabanski, 2000; Kreitinger et al., 2007). Hawthorne and Grabanski (2000) concluded that the best conditions to extract and calculate the F_{rap} of 2- to 5-ring PAHs was 200 bar at 50°C, but other authors used different conditions (Bielská et al., 2013; Cajthaml and Šašek, 2005; Sun and Li, 2005).

The extraction behaviour of individual compounds has been related with their molecular weight (Čvančarová et al., 2013; Szolar et al., 2004). In addition, Hawthorne and Grabanski (2000) observed that the two and three-ring PAHs were associated with F_{rap}, while 5- and 6-ring were mostly in the slower fractions. Regarding the effect of soil properties, Szolar et al. (2004) concluded that OM controls the sorption-desorption behaviour for only some of the field contaminated soils studied, and that no relationship was observed for particle size. Similarly, Hawthorne et al. (2002) did not observe significant correlations between release rates and soil properties (elemental analysis, organic carbon and thermal gravimetric analysis) for the 6 PAHs extracted from manufactured gas plant (MGP) soils, suggesting that other parameters may have influence. Also Bielská et al. (2013) suggest that the properties of OM rather than the OC content have influence on the extractability of PHE and PYR using SFE. On the other hand, the influence of OM and particle size was observed for low molecular weight (LMW) PAHs in spiked samples (Bogolte et al., 2007; Sun and Li, 2005).

Solid phase extraction (SPE) from soil water: in SPE methods, water is used as solvent to extract soil-associate compounds, combined with a compound scavenging resin. The matrix is mixed with an adsorbent and water in batch mode and after physical separation, the resin or the soil are analysed in order to determine the amount that was transferred to the aqueous phase. The principle is keeping the aqueous concentrations close to zero using the resin as an "infinite" sink of desorbed compounds, maximizing the transfer to soil-water interface due to the diffusion gradient created.

One type of resin used is the XAD (styrene divinylbenzene copolymer), which is non-ionic, non-polar and hydrophobic adsorbent, but few studies have used it to predict PAHs bioavailability (Table 3.1 - Table 3.4). In fact, the resin most commonly used is Tenax, which is a solid porous polymer based on 2,6-diphenyl-ρ-phenylene oxide and it was originally used as column packing. SPE methods have been widely used to determine the desorption kinetics of HOCs from soils, due to the high sorptive rate and capacity of the resins to retain these contaminants. A typical operational protocol for calculating the desorption kinetics using Tenax is presented by Cui et al (2013), and a compilation of SPE extraction procedures used in literature is given in Table 3 of Annex II. A simplified Tenax extraction of 6 or 24 h has also been used as a simple method to measure the F_{rap}. One of the advantages of using these methods lies on the fact that resin is easily separated from soil solution, since it floats on the top of aqueous phase, and PAHs can be then extracted using an organic solvent and the resin re-used. Moreover, the resin does not interact with the matrix, it can be used for different types of soil and it's easy to implement a protocol and quality assurance/quality control (QA/QC) procedures. The main disadvantage of this method is that it is time and labour consuming.

Still, some problems may arise for HMW PAHs, due to their low desorption rates and low solubility. For example, Hawthorne et al. (2001) did not calculate the F_{rap} of HMW PAHs in MGP soils using XAD-2 during 120d, because the release was too slow to observe a two phase behaviour. In fact, it was observed in other studies using MGP contaminated soils that Tenax or XAD-4 desorption percentages (at 120 days or 15 days, respectively) decrease with ring size, being negligible for HMW PAHs (Bogan et al., 2005; Li et al., 2005). For desorbed compounds, the fraction available also depended on the ring size, being observed values of 58, 3.3 and 1.1% for NP, PHE and fluoranthene (FLA), respectively (Bogan et al., 2005). Very low desorption percentages (6%) for ∑16 PAHs (profiles dominated by HMW PAHs) were also observed in coke oven soils after 210 d (Ahn et al., 2005). Little is known regarding the influence of soil properties. Unlike what would be expected, on MGP soils a poor positive correlation for the ∑15 PAHs and

organic carbon (OC) content was observed (Bogan et al., 2005). The rate constant of rapid desorption of PYR has been shown to be inversely dependent of OM content in spiked soils aged for 120 days, but no relationship was observed for the desorption percentage (Li et al., 2007). Braida et al. (2004) did not observed a relationship between OC content and the PHE fraction resistant to desorption in 15 spiked soils.

Solubilising agents: some authors stated that for HOCs, simply measure the fraction extracted with water, may not be the most appropriate, since they have low aqueous solubility's and tend to associate with OM and mineral fractions (Hickman and Reid, 2005; Reid et al., 2000b). Therefore, the use of solubilising agents as infinite sink of HOCs desorbed from soils has been suggested as an alternative approach. These agents interact with the matrix by providing a phase into which labile compounds can partition and thus enhancing their extractability.

One of the most frequently used is hydroxypropyl-β-cyclodextrin (HPCD), which was first proposed by Reid et al. (2000b). This is a ring-shaped oligosaccharide comprised of seven α-1,4-linked glucose units, having a high aqueous solubility due to the hydrophilic shell (hydroxyl functional groups). It also has a toroidal shape hydrophobic cavity, with approximately 6.5Å in diameter, which enables the formation of 1:1 inclusion complexes with hydrophobic organic moiety, increasing the organic compounds aqueous solubility (Latawiec and Reid, 2009; Reid et al., 2000b). One assumption of this method is that the size and shape of the organic molecules and HPCD are complementary, which is the case of LMW PAHs, but it is suspected that HMW PAHs may be too large be included in the HCPD cavity, resulting in low extraction efficiency. However, the formation of 2:1 complexes has been suggested for HMW compounds (Latawiec and Reid, 2009).

Extraction with HPCD has been used to calculate desorption kinetics (Rhodes et al., 2010; Sabaté et al., 2006), though the typical operational protocol consists in single extractions. Most studies performed a 20h extraction (Table 4 of Annex II), which is considered the weakly bound fraction, corresponding to F_{rap}. Yet, other extraction parameters varied between studies (e.g. concentration of HPCD, ratio soil: solution). Besides, after extraction, the solution is separated from soil (filtration or centrifugation) and some authors analyse the soil pellet whereas others analyse the HPCD solution, which has been suggested as being one of the causes of the different results obtained (Gomez-Eyles et al., 2012; Sabaté et al., 2006; Stokes et al., 2005). Latawiec and Reid (2009) concluded that in spite of being a good predictor, it can be more expensive than other methods such as Tenax. Still, using solubilising agents may be fast and of easy operation.

Due to the working principle, it is expected to have higher extractabilities than those obtained with SPE methods, being closer to mild solvent extraction. However, the extraction of ∑15 PAHs from a gasworks' site was 0.99% using HPCD, which was higher than using 1% MeOH or BuOH (0.16 and 0.13% respectively), but much lower than using 50% MeOH (5%) (Bergknut et al., 2007). On the other hand, higher extracted percentages were observed in MGP soil (ranging between 20 and 29% for the ∑15 PAHs), and the extraction decreased with increasing ring number (77% for NP and 10% for BghiP), being the relationship with log K_{OW} statistically significant (Papadopoulos et al., 2007a). Also, in a creosote contaminated soil, the extractability was higher than 90% for 3-ring PAHs and FLA, between 80-90% for PYR, BaA and CRY, but 5- and 6-ring PAHs were not recovered (Sabaté et al., 2006). The influence of soil properties in PAHs extractability using HPCD has been widely studied, but results are not unequivocal. Several studies (Hickman and Reid, 2005; Papadopoulos et al., 2007a; Rhodes et al., 2008a; Rhodes et al., 2008b; Swindell and Reid, 2006) found lower extractabilities in soils with high OM or black carbon (BC) contents. Moreover, the PAHs extracted after 155h with HPCD in roadside soils ranged between 1 and 5%, in which the low values were attributed to the presence of soot (Johnsen et al., 2006). On the other hand, Patterson et al. (2004) concluded that NP extractability was related to dissolved OC rather than to texture or OM content in spiked soils. Rhodes et al. (2010) did not observe any trend between desorption rate constants and soil type, for PHE spiked soils.

Surfactants have also been used to increase the solubility of HOCs. Surfactant molecules aggregate forming micelles with a hydrophobic core, which incorporate the HOCs (Ehlers and Loibner, 2006; Guha et al., 1998). The surfactant molecules penetrate into pores and the solid phase causing intrasorbent swelling of the soil, therefore resulting in an increased concentration gradient at the soil-water interface and matrix diffusivity of PAHs (Yeom et al., 1996). The amount of PAHs solubilised depends on the properties of the compounds and the structure and concentration of surfactant (Guha et al., 1998; Thiele-Bruhn and Brümmer, 2004). The critical micelle formation concentration is particularly important, being the PAH solubilisation proportional to the surfactant dose above this concentration (Thiele-Bruhn and Brümmer, 2004). The procedure is similar to the one used for HPCD, but different conditions and several surfactants have been tested (Table 4 of Annex II): Genapol88, Synperonic LF/RA 30 (Thiele-Bruhn and Brümmer, 2004); Brij 700 (Latawiec and Reid, 2009); Tween-80 (Bergknut et al., 2007). Extractability of PAHs with surfactants seems to be higher than with HPCD. For instance, Tween-80 extracted 6.3% of the total PAHs (∑15) from a gaswork site, whereas HPCD extracted only 0.99% (Bergknut et al., 2007). Moreover, Thiele-Bruhn and Brümmer (2004) found that the used

surfactants dissolved up to 71% of each individual PAH (3- to 6-ring), in different industrial soils. Even so, these authors observed that the surfactant-extractable PAH concentration decreased with decreasing water solubility and increasing molecular weight of compounds.

Persulfate Oxidation: Cuypers et al. (2000) proposed a rapid method to predict PAHs bioavailability based on persulphate ($S_2O_8^{2-}$) oxidation. The principle is that organic matter is oxidized by sulphate radicals formed during the heating of persulphate. The persulphate oxidation affects the expanded organic matter, which contains the readily available fraction (low sorption affinity with PAHs and lower resistance to microbial degradation), in opposition to condensed organic matter which contains the non-available fraction. Little is known about the behaviour of PAHs in soils when using this method since few studies have been made (Table 3.1 - Table 3.4). Even so, it was observed that the amount of HMW PAHs extracted by this method in creosote-contaminated soils, was insignificant in opposition to LMW PAHs (Juhasz et al., 2005).

3.2.2 Biomietic methods

Biomimetic (or passive sampling) methods give the concentrations actually or currently available and are related with the chemical activity of contaminants (Reichenberg and Mayer 2006). The chemical activity of a compound is logarithmically related to its fugacity and linearly related to its freely dissolved concentration (Mayer et al., 2003). Hence, passive samplers include all the techniques that measure the free flow of contaminants from the matrix to the sampler in a non-depletive manner, since only a minor portion of the analyte is removed from the matrix (Brand et al., 2013; You et al., 2011). These methods allow for a selective partitioning and adsorption of analytes to a surrogate phase in order to simulate desorption from soil and, after achieving the equilibrium, to estimate the bioavailable concentrations by calculating the freely dissolved concentration in pore water (C_{free}). The exchange kinetics between sampler and pore water can be expressed by a first order one-compartment model: $C_{PS}(t)=C_{free}*(k1/k2*(1-e^{-k2*t}))$, were C_{PS} is the concentration in the passive sampler and k1 and k2 the uptake and desorption rate coefficients, respectively.

It is assumed that, at equilibrium conditions, the concentrations in the different compartments (sampler, pore water and soil) are proportional to each other. This assumption is closely related to the basis of the Equilibrium Partition Theory (EqPT), which is the most widely accepted theory concerning chemical's uptake by organisms in sediments, and considers that bioavailability of HOCs is controlled by equilibrium partitioning between sediment OC, water and the lipids of

organisms. This theory has been also applied to soils, even though some of the assumptions are normally not verified in this case. For example, calculations of C_{free} may not be appropriate to describe exposure in dry media such as soil, since they are usually not saturated and, therefore, contaminants in pore water are not in equilibrium (Reichenberg and Mayer, 2006).

In EqPT it is assumed that OC fraction (f_{OC}) and organic carbon-water partition coefficient (K_{OC}) are the most important factors that determines soil-water partition coefficient (K_d), which is given by $K_d = f_{OC} * K_{OC}$. According to this theory, it is possible to calculate the C_{free} ($C_{free}=C_{soil}/(f_{OC}*K_{OC})$ once the concentration in soil (C_{soil}) is known. Concentrations in an organism (C_{org}) can be further estimated by the relationship between pore water and the bioconcentration factor (BCF), using the following equation: $C_{org} = BCF*C_{free}$. BCF is, therefore, the partition of the compound between water and the lipid content of the organism and it is generically assumed to be equal to K_{OW}. Moreover, at steady state concentrations in an organism (C_{org}), lipid (lip) normalized, are related to the OC normalized C_{soil} through a constant factor, which is the biota-to-soil accumulation factor (BSAF): $BSAF=(C_{org}*f_{OC})/(C_{soil}*lip)$. This constant factor is independent of the soil type, species or compound properties and it is expected to be independent of K_{OW} (Krauss et al., 2000; Ma et al., 1998; Sijm et al., 2000). Yet, EqPT is not always valid and deviations are likely to occur due to several factors such as sequestration of pollutants in the soil (Sijm et al., 2000). When comparing with other compounds, for instance, PAHs are normally more strongly adsorbed to soils and have a slower desorption rates (Jager et al., 2003; Krauss et al., 2000). In addition, Ter Laak (2006b) showed that EqPT models can predict sorption coefficients of spiked soils (fresh or aged) but not of field contaminated soils, where C_{free} are normally lower than predicted. Besides, these models normally use generic K_{OC} values and it has also been shown that they do not reflect the behaviour of naturally contaminated samples, since factors such as the quality of OM may have a strong influence (Jonker et al., 2007). A direct method to measure the predicted bioavailable fraction is the determination of HOCs present in soil pore water, but the problem of this approach is that the levels are often the below detection limit and the presence of dissolved organic matter may affect the measured concentrations. Biomimetic methods are indeed the best approach to refine the estimations based on EqPT.

The use of passive samplers allow the determination of specific K_{OC} values, derived from C_{free} ($K_{OC}=C_{soil}/(C_{free}*f_{OC})$. The C_{free} is based on the measurement of the equilibrium concentration in the sampler (C_{PS}) and the sampler-to-water partition coefficient (K_{PS-W}): $C_{free=}(C_{PS}/K_{PS-W})$ (Mayer et al., 2003). Once the C_{free} is known, it's possible to estimate the C_{org} as described before, assuming that the BCF values are equal to K_{OW} (Jonker et al., 2007). Accurate measurements of C_{free} and K_{OC} with

biomimetic methods require the sampler to be in equilibrium and soil concentration to be unaffected by the sampler (ter Laak et al., 2006b). Accurate K_{PS-W} calculations are also needed, including the use of the same experimental conditions of sampling. If sampling is not performed at equilibrium, kinetic data is needed to estimate C_{free} and rate constants, by using a one or two-phase uptake model (Mayer et al., 2003; ter Laak et al., 2006b). Another approach when equilibrium is not reached is to use deuterated PAHs that allows correcting and calculating the data (Ghosh and Hawthorne, 2010).

The biomimetic methods can also be used as a surrogate of the organisms, i.e. C_{PS} can be assumed to be an estimation of C_{org}, or EqPT can be applied by calculating the passive sampler-to-soil accumulation factor - $PSSAF=(C_{PS}*f_{OC})/(C_{soil}*lip)$ - which theoretically would be equivalent to BSAF.

***Solid Phase Microextraction (SPME*)**: Several studies have established SPME as a reliable method to assess the C_{free} for several HOCs, including PAHs (ter Laak et al., 2006b). This approach uses a fiber with a silica core that contains a thin film of organic phase (normally polydimethysiloxane – PDMS) to extract HOCs from the aqueous phase. Compounds are absorbed via diffusion into the organic phase, with no competition between compounds or saturation of the layer, until the thermodynamic equilibrium is established (Ehlers and Loibner, 2006; You et al., 2011).

Both injector type or disposable SPME may be used, though the present study will be focused on the latter since it's much more flexible, practical and cheap (fibers can be regenerated) (Jonker et al., 2007). This method is normally called matrix-SPME, because sampling is achieved by immersing the fibre in an aqueous suspension of soil with gentle shaking (Table 5 of Annex II). After exposure, the analytes are extracted from fibers using solvent extraction and concentrations in fibers are then transformed in C_{free} using predetermined fiber-to-water partition coefficients (K_{SPME-W}). Some of the disadvantages of this method (Cui et al., 2013; Jonker et al., 2007; You et al., 2011) are: fiber stability (both physical and chemical) and sensitivity; SPME may be influenced by several parameters (temperature, etc...); fiber surface fouling (may affect uptake kinetics or increase sorption capacity); matrix effects; too long sampling times (it can take weeks or months to reach equilibrium); and difficulty to define a universal equilibration time. However, the sensitivity and sampling times may be adjusted by adjusting the surface-volume ratio of sampler, mass of sample and size of passive sampler (ter Laak et al., 2006a).

Ter Laak et al. (2006b) observed that uptake kinetics of seven PAHs (3- to 6-ring) in spiked soils, aged for 3 weeks, was different from field contaminated soils, with equilibrium reached in 3 days in the former and several months in the latter. The reason given was that in spiked soil desorption from soils is not the rate-limiting step, whereas in field contaminated soils it is. A similar conclusion was obtained by Jonker et al (2007): differences in uptake kinetics of fibers (from 1 to 10 weeks to reach the equilibrium for several MGP soils) were due to differences in PAH desorption. However, the authors argue that the different behaviours between samples are most likely to be related to sources rather than soil characteristics. On the other hand, other authors observed that OC/OM inversely affected the C_{free} of both PHE and PYR in spiked soils (Styrishave et al., 2008; Yang et al., 2009). Variations due to aging (up to 553 days) were not very clear for 3- to 6-ring PAHs (ter Laak et al., 2006b; Yang et al., 2009), but in another study conducted with PYR, a decrease of C_{free} with soil contact, depending of the OM content was observed (Styrishave et al., 2008).

Polyoxymethylene solid phase extraction (POM-SPE): POM is a polymer that contains a repeated polar group and it has been used to determine the partition of HOCs in sediment systems. It has only recently been tested to predict bioavailability in soils and only one study used this polymer to predict PAHs bioavailability (Table 3.1 - Table 3.4). Advantages of this method include their stability, resistance to organic solvents and low propensity to fouling, whereas the main disadvantages are the long sampling times and large volumes of sample needed (Cui et al., 2013). The operational procedure is similar to SPME, but the soil: water ratio is normally higher. For example Gomez-Eyles et al. (2012) used 3g of soil to 110 mL of 0.01M $CaCl_2$ (76 µm thick POM, exposure time of 28 days at 20°C, in a shaker at 150 rpm).

Extraction disks: C18 membranes have been used as passive sampler, but few studies have used them to predict availability of PAHs (Table 3.1- Table 3.4). As for the other passive samples referred previously, the method consists in the suspension of a disk in soil slurry and a further extraction of contaminants from the sampler using a solvent. The main disadvantage is that due to the high volume of the hydrophobic C18, a volume of water higher than in other materials such as composite membranes or SPME is required.

Krauss and Wilcke (2001) used a soil:solution ratio of 10:50 mL and a 50 mg C18 disk with a gentle agitation performed every second day for 15 days. In the other study, it was used a soil: solution ratio of 1:30 mL and also one C18 disk, shaken in an end-over-end for 14 days (Tang et

al., 2002). Albeit being performed in spiked soils, the last study did not prove that the equilibrium was reached. Krauss and Wilcke (2001) observed that after 15 days, at 20°C, the calculated PAH concentrations in disks were 79±12% of the theoretical steady state but if disks were exposed at 40°C this value rises to 92±6%. These calculations were performed for 20 PAHs in one urban soil and the authors assumed that, for the other 24 soils, the steady-state was also achieved using these conditions. The C18 disks only extracted 0.1% of individual PAHs and the partition coefficient between disks and soils (K_{disk}) varied widely, reaching 0.41 (for ACE and fluorene) and decreasing with K_{OW}, probably due to desorption rate limitation.

Composite Membranes: Semi-permeable Membrane Devices (SPMDs) are made of low-density polyethylene tubes with octanol or triolein inside and are commonly used as surrogate to predict availability of HOCs to aquatic organisms. Recently, Tao et al. (2008a), have used triolein embedded cellulose acetate membranes (TECAMs) to predict bioavailability of PAHs in soil samples. Later, Li et al (2010) proposed a new type of composite membrane (cellulose acetate membranes embedded with petroselenic acid) to specifically predict the availability of HOCs to plants, since this compound is the major component of plant lipids. However, these membranes were not yet tested in soil samples and will not be discussed in the present work.

In all cases, membranes mimic the passive transfer between the organism's fat tissues and dissolved HOCs (excluding contaminants that are attached to particles or associated with colloidal material) which are available to pass the membrane and accumulate in the internal lipid (Cui et al., 2013; MacRae and Hall, 1998). In the case of TECAMs, since the triolein is uniformly mixed with the cellulose acetate, the resistance of mass transfer to the lipid is minimal, compared with SPMD.

The method consists in the exposure of membranes to soil (without agitation), and then their extraction with a solvent. The soils are normally kept at 60-70% of water holding capacity (similar conditions to organism's uptake), which is one of the advantages of composite membranes compared with the previous described methods. However, a depletion of PAHs nearby the sampler may occur due to the diffusive transport of small and more mobile PAHs. Other problems may be the slow sorption kinetics (it can take weeks of exposure), the high quantity of the sample (to obtain acceptable analytical sensitivity) and the problem of fouling of membranes. The use of TECAMs may reduce the high sampling times required by SPMDs, since the cellulose dialysis membranes have hydrophilic hydroxyl groups which can decrease the surface tension between membrane and soil solution and consequently have a fast exchange kinetics (Li et al., 2010; Tao et

al., 2009). Other advantages of TECAMs include having a large contact area (1 mL triolein on approximately 15000 cm^2 surface area of cellulose acetate), being easily established in laboratory and being inexpensive (Tao et al., 2008a; Tao et al., 2008b).

Bergknut et al, (2007) concluded that PAH profiles in SPMDs had relatively high proportions of small PAHs, being one possibility that steady state was reached only for the 3-ring PAHs during the 3-week exposure. Another possibility is the referred rapid depletion of the reservoirs of these LMW PAHs closer to membranes. Tao et al. (2009) observed that the sampling process of 4 PAHs (NP, PHE, PYR and BaP) by TECAMs in naturally contaminated soils corresponded with first-order kinetics model and steady state was reached after 7 day exposure (being the time for 95% equilibration of 9.3 h for NP to 18.5h for BAP).

Tao et al. (2008a) found that the amount of the same 4 compounds that accumulate in TECAMs, in field contaminated samples, was not very different for each one (0.27±0.03, 0.16±0.01, 0.21±0.03, and 0.33±0.08% of the total amount for NP, PHE, PYR and BaP, respectively). In another study (Tao et al., 2009) also using field contaminated soils, the relationships between concentrations of PAHs in TECAMs and soils (K_{TECAM}) varied for each compound according to their K_{OW} (53, 52, 41, and 8.1 for NP, PHE, PYR, and BAP, respectively), suggesting that the desorption limitation is the most probable reason. However, these factors were much lower in spiked soils: 0.47, 2.84, 1.24 and 0.33 for NP, PHE, PYR and BAP, respectively (Tao et al., 2008b). Nevertheless, no explanation is given for these contradictory results, since it was expected to observe higher K_{TECAMs} in spiked soils than in field contaminated. Moreover, in this study no linear relationship between log K_{TECAM} and log K_{OW} was observed, but if excluding NP it seems that there was also a decrease with log K_{OW}. Yet, a significant positive linear relationship (R^2=0.988, $p<0.01$) was observed between log $K_{TECAM-W}$ (partition coefficient between TECAM and water) and log K_{OW}. In the same study it was also observed that the quantities of the 4 PAHs accumulated by TECAMs were negatively related to OM and positively related to dissolved OC and an effect of aging was also observed (Tao et al., 2008b).

3.3 STATE OF THE ART OF THE PREDICTION OF PAH AVAILABILITY BY CHEMICAL METHODS

In order to understand the meaning of the chemical assays and in an attempt to use them as alternative to bioassays, several studies investigated their correlation with biological assays. The approach normally used is either the comparison of how they correlate with the amount of HOCs degraded by microbes, accumulated by biota (earthworms, plants) or the response of ecotoxicological tests. Bioavailability is organism's specific, due to differences in animal behaviour

or different metabolic fates in organisms, which result in dissimilar uptake and/or elimination rates (Ten Hulscher et al., 2003). For example, availability of HOCs to microorganism is unlikely to be related with its availability to higher organisms (Bogan and Sullivan, 2003). Moreover, although several studies have concluded that aging affects bioavailability, there are some differences between organisms. Yet, several studies conclude the feasibility of a chemical method by comparing it with other studies where different bioassays were used (e.g. Khan et al., 2011). Therefore, the further discussion will be separated between microbial degradation, earthworm and plant's accumulation and ecotoxicological tests. Some difficulties were faced, since similar methods may use different conditions, or even different target species within an organism group, which can give different results, making it difficult to evaluate them. The timescale considered will also affect the amounts of PAHs accumulated or degraded. Another problem when comparing different methods is the data presentation and different normalization approaches used.

In the case of non-exhaustive extractions, direct comparisons are typically made by performing a linear regression between the extracted percentage as a function of the biodegraded or accumulated percentage. However, some authors suggest that it's possible to predict concentrations in organisms by applying the EqPT calculations, i.e. based on C_{free} (Gomez-Eyles et al., 2012; van der Heijden and Jonker, 2009). In this case, C_{free} is determined by dividing the OC-normalized concentration extracted from soils (C_{ext}) by the generic organic carbon-water partition coefficient (K_{OC}) derived from K_{OW}: $C_{free}=C_{ext}/(K_{OC}*f_{OC})$. Other authors (Ten Hulscher et al., 2003) also suggest an approach based on EqPT models, but by calculating the BSAF based on desorbing fraction (C_{ext}) instead of total concentrations $BSAF=(C_{org}*f_{OC})/(C_{ext}*lip)$.

Outputs of biomimetic methods can be used in several ways. If passive samplers are considered a surrogate of the organisms, a direct comparison of concentrations can be made ($C_{PS}=C_{org}$), while also performing a linear regression between the concentration in the sampler as a function of the concentration accumulated, with or without lipid normalization. A direct comparison of the passive sampler-to-soil accumulation factor with the BSAF can also be made. If using the C_{free} it's possible to estimate the C_{org} as previously described (section 2.2) or to make a direct comparison of C_{free} with C_{org}.

A compilation of literature results of comparisons between chemical methods and biossays are presented in Table 3.5 - Table 3.7. In some cases, the relationship was performed by calculating the ratio between chemical method and bioassay, whereas in other cases it represents the slope of the line of the best fit. Therefore, when the relationship is presented, it either represents the ratio of chemical method vs. bioassay, the slope of the linear correlation of the extracted fraction

by chemical method as a function of the fraction biodegraded or accumulated, or the residual fraction after bioassay, as a function of the residual fraction after chemical extraction. Yet, results are always interpreted in the same way: a slope higher than one means that chemical methods overpredict biodegradation and if it is lower than one there is an underestimation.

3.3.1 Microbial degradation

Microbial degradation is one of the most important processes for PAHs declining in soils, due to their catabolic activity (Haritash and Kaushik, 2009; Ling et al., 2009). Degradation parameters of contaminants are used as indicators of bioavailability to assess the potential of bioremediation, for instance. However, the availability of the sorbed HOCs to microorganisms is still not well understood. Yet, it is known that two different processes are involved: the transfer of contaminant from the soil to the aqueous phase (physical or chemical) and the metabolism of the compound (biological) (Semple et al., 2003). Regarding the latter, most important aspects were reviewed in the study of Haritash and Kaushik (2009). However, it is important to highlight that it is species and compound's specific and that soil properties such as pH, humidity and its nutrients' availability rule the microbial activity (Haritash and Kaushik, 2009; Li et al., 2005; Sabaté et al., 2006). Yet, the effect of mixtures on biodegradation is not well known, since some studies refer that the presence of some compounds may inhibit the degradation of others, whereas other studies refer the stimulation of degradation of PAHs present in mixtures (Haritash and Kaushik, 2009).

Microbial degradation has the similar biphasic profile of desorption: an initial fast biodegradation phase, where the rate of PAHs removal is suggested to be primarily limited by microbial degradation kinetics, and a second slow phase, in which biodegradation should be limited by desorption rates (Allan et al., 2006; Doick et al., 2005; Haritash and Kaushik, 2009; Rhodes et al., 2008b). However, it is suspected that microbes not only utilise contaminants present in the liquid phase (the ones that are rapidly desorbed from particles) or in submicrometric particles dispersed in the aqueous phase, but they are also able to degrade the sorbed contaminants, suggesting that other factors may affect the relationship between biodegradation and desorption rates (Braida et al., 2004; Haritash and Kaushik, 2009; Semple et al., 2003; Yang et al., 2009). Moreover, it is known that some microbes can produce biosurfactants, enhancing the desorption rates and, consequently, the bioavailability of PAHs (Haritash and Kaushik, 2009). Nevertheless, it is accepted that the availability to microorganisms depends primarily on the speed of desorption that, in turn, depends on the soil type (Hickman

and Reid, 2005), compound (Juhasz et al., 2005) and aging (Rhodes et al., 2008b; Tang et al., 1998).

Regarding soil properties, higher microbial degradation in soils with lower OM and clay mineral contents was observed (Hickman and Reid, 2005; Rhodes et al., 2008a; Rhodes et al., 2008b; Thiele-Bruhn and Brümmer, 2004). In addition, the presence of BC has been shown to reduce mineralization from 70.8 to 17.2% (Rhodes et al., 2008a). On the other hand, a high content of fulvic acids (soluble fraction of organic matter) resulted in a higher rate of PAHs degradation, whereas the sequestration of these compounds occurs mainly in the humin fraction (Bogan and Sullivan, 2003). Other authors concluded that OM content was not the only factor affecting microbial availability of PAHs: Yang et al. (2009) suggest that soil porosity was an important factor (reduced porosity inhibited the sequestration in soils with higher OC contents). Bogan and Sullivan (2003) suggest that in addition to micropore volume, the surface area may have some influence.

A wide range of biodegradation rates of different naturally contaminated soils can be found in literature, most of them using indigenous microorganisms: the sum of ∑16 PAHs decreased 16 to 72% in four industrial soils and 33±5.4 to 83.4±8.7% in two soils from MGP, both after 6 weeks of inoculation with catabolically active microorganisms in addition to indigenous (Hickman et al., 2008); in 10 contaminated amended soils from gasworks and coking plants, the concentration of PAHs (∑15) decreased 53 to 93% after 74 weeks (Thiele-Bruhn and Brümmer, 2004); in MGP soils, the decrease for the ∑15 PAHs ranged from 18 to 30% after 8 weeks of incubation (Papadopoulos et al., 2007a); the PAH (∑13) degradation in 4 industrial soils after composting for 8 weeks ranged from 64 to 96% (Cajthaml and Šašek, 2005); but only from 0.3 to 9.6% (∑12) in 11 industrial soils that were incubated for 245 days (Bernhardt et al., 2013). Moreover, other authors suggest that naturally contaminated soils may not be biodegradable, especially if they have a high percentage of HMW PAHs. For example Szolar et al. (2004) studied 8 industrial soils and 5 of them showed no biodegradation of the 8 PAHs with 4- and 5-rings studied, after inoculation with PAH-degrading bacteria during 14 weeks. In creosote-contaminated soils, after 16 week incubation with indigenous microbes, 89 to 99% of 3 ring-PAHs were degraded, 21 to 79% of the 4-rings and no degradation of 5- and 6-rings PAHs was observed (Juhasz et al., 2005). In coke works' soils, the biodegraded % after 6 weeks ranged between 0.7 (for BAP) and 53% (for NP) using an inoculum of PAH-degrading bacteria (Stokes et al., 2005). Similarly, in MGP soils only LMW PAHs, especially 2 and 3 ring PAHs, were removed at a great extent during 1 year bioremediation with indigenous microbes, whereas the 5 and 6 ring PAHs were not degraded (Hawthorne and Grabanski, 2000). In

another study conducted in MGP soils, the biodegradation ranged from 77% for NP to 6% for dibenzo(a)anthracene, being the relationship with log K_{OW} statistically significant (Papadopoulos et al., 2007a). Other authors also observe that the rate of degradation decreases when the number of rings increases (Li et al., 2005; Sabaté et al., 2006; Szolar et al., 2004). The main reason is believed to be the low desorption rates of HMW PAHs which tend to be recalcitrant in soils. However, it is known that only a limited number of bacteria can grow in pure cultures of PAHs with 5 or more rings, and therefore the ability of the microbial communities for their degradation is low (Haritash and Kaushik, 2009). Other reason may be due to the biological factors such as the slow transport of HMW PAHs over microbial cell. In addition, it has also been demonstrated that some microorganisms may have active efflux mechanisms which could play an important role in controlling intracellular and membrane concentrations, especially for PAHs with higher K_{OW} (Bugg et al., 2000). Hence, the low biodegradation rates of HMW PAHs may be either due to the low desorption rate from soils or due to the inability of communities to degrade these compounds.

3.3.1.1 Chemical methods for assessing microbial availability

Based on the principle that microbial availability is first governed by contaminant mass transfer from the solid phase to the aqueous phase, several non-exhaustive extractions have been proposed to predict bioavailability to microbes (Swindell and Reid 2006). Regarding mild solvent extraction it becomes very difficult to arise any conclusion about the feasibility of these methods due to the small amount of tested compounds, the differences in operational procedures and the fact that most studies were performed in one single spiked soil (Table 3.5 and Table 2 of Annex II). A BuOH extraction with agitation was addressed as the most appropriate solvent for predicting microbial degradation of PHE, when comparing with other solvents (Kelsey et al., 1997). Further studies conducted with spiked soils also found good correlations between BuOH extractions and PHE or PYR biodegradation, but others did not found any significant correlation (Table 3.5). Yet, a significant correlation was observed for the ∑15 PAHs in industrial soils, not only for BuOH but also using other solvents. Regarding the relationship between the two approaches (Table 3.5), using either pure solvents or mixtures showed correlations close to 1 for PHE and PYR in spiked soils, but a slight overestimation was observed in a soil that was aged for a longer period than the others. Similarly, BuOH or MeOH extractions showed an overestimation for the ∑15 PAHs in industrial soils, but using 50% EtOH the relationship was close to 1. Other studies, also using naturally contaminated samples but reporting results for individual compounds instead of the sum, found that this overestimation increased with K_{OW} of compounds (Juhasz et al., 2005;

Latawiec and Reid, 2009). Even more, Juhasz et al. observed a significant underestimation of LMW PAHs (being lower when using pure solvents) and an overestimation of HMW PAHs (being higher using pure solvents) in cresote contaminated soils. Macleod and Semple (2003) used a sequential extraction and concluded that the first step (50% MeOH) underestimated the availability of PYR in aged soils, whereas the second step (BuOH) gave an overestimation.

The use of SWE was tested to predict PHE biodegradation in 2 dissimilar soils at 3 different contact times, but results are not very consistent (Latawiec et al., 2008). Overall, the authors did not found significant differences between SWE, at 160°C (10 min), and biodegradation, although two of the six comparisons made showed incoherent results. Further, these authors used the same method to extract several PAHs from spiked and naturally contaminated soils and found that availability to microorganisms was underestimated (as high as around 70% for 3 ring PAHs) (Latawiec and Reid, 2009). However, these authors observed that the extraction efficiency decreased as the log K_{OW} increased, being the extractions analogous to the bioavailable fraction.

Very good linear correlations have been obtained using SFE either for the ∑13 PAHs or for individual compounds in naturally contaminated soils (Table 3.5). Yet, the conditions applied were different for each study and few dissimilar soils were tested. Hawthorne and Grabanski (2000), for instance, obtained the best results with the mildest sequential SFE condition (60 min, at 120 bar and 50°C) but, in another study, Hawthorne et al. (2001) used a single extraction and found that 20 min extraction (200 bar and 50°C) successfully simulated a 147-day field bioremediation for the 20 PAHs. Other study conducted by Cajthaml and Šašek (2005) concluded that the best compromise between F_{rap} and biodegradation of ∑13 PAHs in 4 dissimilar soils was achieved by changing the pressure to 300bar. These authors observed differences in the coefficients of correlation of PAHs groups, with r^2 ranging from 0.74-1.0 for 3-ring PAHs, from 0.80-1.0 for 4-ring and from 0.27-0.99 for 5- and 6-ring PAHs. There is also increased underestimation as the molecular weight increases. On the other hand, Hawthorne et al. (2001), stated that the method tends to overestimate availability of middle molecular weight PAHs (PYR-CRY). Szolar et al (2004) found that the first two phases of a sequential SFE roughly estimates biodegradation, even though it does so with a tendency for underestimation. In spite of all this, it could be a promising method even though it is not easy to implement, since few laboratories have the required technology.

Table 3.5 - Comparison between microbial degradation assays and several chemical methods.

Method	Compounds	Correlation (r^2)	Relationship	Number of soils; aging[a]	References
BuOH	NP	NS[b]	-	1, 68d	Kelsey and Alexander, 1997
BuOH	PHE	-	1.05±0.02	1, 120d	Kelsey et al., 1997
BuOH	PHE	0.96	1.63	1, 322d	Reid et al., 2000b
BuOH	PHE; PYR	0.97; 0.99	0.952; 0.998	1, 47d	Liste and Alexander, 2002
BuOH	PHE; PYR	NS[b]	-	6, 120d	Bogan and Sullivan, 2003
BuOH	∑15	0.73[c]	2.41	10	Thiele-Bruhn and Brümmer, 2004
MeOH	∑15	0.76[c]	2.80	10	Thiele-Bruhn and Brümmer, 2004
50% MeOH	PHE	-	1.04±0.07	1, 120d	Kelsey et al., 1997
50% EtOH	PHE	-	1.10±0.09	1, 120d	Kelsey et al., 1997
50% EtOH	∑15	0.85[c]	0.95	10	Thiele-Bruhn and Brümmer, 2004
SFE	∑8	-	0.99[d]	8	Szolar et al., 2004
SFE	∑13	0.81-0.99	-	4	Cajthaml and Šašek, 2005
SFE	20 PAHs	0.93	-	1	Hawthorne and Grabanski, 2000
SFE	20 PAHs	0.96	-	1	Hawthorne et al., 2001
XAD2	20 PAHs	0.96	-	1	Hawthorne et al., 2001
XAD4	NP	NS[b]	-	5, 2d	Patterson et al., 2004
Tenax	PHE	0.657	0.81[d]	15, 190d	Braida et al., 2004
HPCD	NP	0.917	-	5	Patterson et al., 2004
HPCD	PHE	0.964	1.07	1, 322d	Reid et al., 2000b
HPCD	PHE	0.89	1.11	4, 100d	Allan et al., 2006
HPCD	PHE	0.88	0.99	4, 37d	Hickman and Reid, 2005
HPCD	PHE	0.13-0.95	0.079-1.12	4, 100d	Rhodes et al., 2008a
HPCD	PHE	0.95-0.99	0.99-1.08	4, 100d	Rhodes et al., 2008b
HPCD	PHE	0.97-1	0.89-1.08 (1.04)	2, 139d	Doick et al., 2006
HPCD	PHE	0.96-0.98	1.01-1.05	4, 100d	Papadopoulos et al., 2007b
HPCD	PHE	0.89	1.18	4, 100d	Rhodes et al., 2010
HPCD	13 PAHs	0.77	0.88[d]	1	Juhasz et al., 2005
HPCD	∑14 PAHs	0.94-0.98	0.93-1.07	6	Papadopoulos et al., 2007a
HPCD	16 PAHs	0.94-0.99	0.73-1.4[d]	6	Hickman et al., 2008
HPCD	∑24 PAHs	0.85-0.96	0.8-1.0 (0.92)[d]	2	Doick et al., 2005
HPCD	16 PAHs	0.79-0.99	0.84-0.93[d]	2, 12m	Stokes et al., 2005
	20 PAHs	0.986	1.032[d]	1	
Surfactants	∑15 PAHs	0.71-0.73[c]	1.31-1.36	30	Thiele-Bruhn and Brümmer, 2004
Persulfate	13 PAHs	0.86	1.06[d]	1	Juhasz et al., 2005
Persulfate	∑16 PAHs	0.95	0.97[d]	14[f]	Cuypers et al., 2000
SPME	PHE	0.483-0.908[c]	-	5, 120d	Yang et al., 2009

[a]Number of soils with dissimilar properties tested and maximum aging period in days (d) or months (m) for spiked soils; [b]Not significant; [c]correlation coefficients (r); [d]based on remaining fractions;[f]7 soils+7 sediments

The PAH extraction using XAD2 during 2.2 days correlated well with a 147-day field bioremediation of MGP soils (Table 3.5). However, the authors found some differences between compounds and stated that, regarding middle molecular weight PAHs (PYR-CRY), the method tends to overestimate availability, as observed for SFE. Another study found poor correlations between NP mineralization and a 20h extraction with XAD4 in spiked soils (Table 3.5). Even though the SPE using Tenax showed to provide an estimation of the amount available for biodegradation in sediment samples, few studies were conducted in soils (Table 3.1). Bernhardt et al. (2013) concluded that residual concentration of ∑12PAHs in industrial soils after biodegradation was similar to the non-extracted concentration, but the relationship was not shown. Similar results were observed by Braida et al (2004), which calculated the relationship between the PHE fraction resistant to desorption and the one resistant to biotransformation after 30 days, in 15 spiked soils (Table 3.5). However, this study emphasizes that the decline in mineralization of aged PAHs, at late times, is rate-limited by desorption. The authors also stated that other organisms capable of producing larger amounts of surfactants would be less dependent of desorption rate. In fact, another study conducted by Li et al (2005) found that the fraction of ∑12 PAHs resistant to desorption was greater than the fraction resistant to biodegradation, but no correlations were calculated. These authors suggest several explanations: the production of biosurfactants by microorganisms that enhance the solubilisation of compounds; microorganisms are able to "use" contaminants directly from soil particles; and they may have access to contaminants in the film layer. These authors also observed that the resistance to both desorption and biodegradation increases with ring size.

HPCD is considered a good estimator of PAH bioavailability to microorganisms, since it conceptually mimics the mass transfer process governing microbial bioavailability, mainly due to the ability of microorganisms to produce surfactants. Indeed, several studies found strong correlations and slopes close to 1 between HPCD extractions (around 20h, corresponding to the F_{rap}) and microbial degradation of PHE, in dissimilar spiked soils (Table 3.5). Though, Rhodes et al. (2010) calculated the desorption kinetics of PHE and concluded that the F_{rap} occurred from 0 to 6h.

Deviations to the predictability using HPCD may derive from the presence of BC, since the increase content affected the linear correlations and the relationship: both decreases (Table 3.5) as the BC content increases from 0 to 5% (Rhodes et al., 2008a). Other authors (Doick et al., 2006; Papadopoulos et al., 2007b) tested the method to predict the availability of PHE in co-contaminated spiked samples and found that it still provided a good estimation for PHE. The

estimation of biodegradation using HPCD also proved to be applicable to dissimilar naturally contaminated soils, considering several PAH compounds (Table 3.5). Even considering each one of the 24 PAHs investigated, Doick et al. (2005) stated that the indigenous microflora has degraded a quantity equal to that extracted by the HPCD. However, several other studies refer differences between compounds, generally with a slight overestimation (slope around 2) of the biodegradation endpoint for HMW PAHs probably due to the desorption limitation of these compounds (Juhasz et al., 2005; Papadopoulos et al., 2007a). In the specific study of Hickman et al. (2008), results are given by ring classes for two different types of soil, and it was observed that slopes were more variable for HMW PAHs: for 2- and 3-ring the slopes were between 0.75 and 0.98; for 4-ring they were 0.98 and 1.38, whereas for 5- and 6-ring they were 0.78 and 1.80. Similarly, correlations were better for LMW PAHs: the r^2 was 0.95 and 0.97 for 2- and 3-ring; 0.93 and 0.97 for 4-ring; and 0.83 and 0.98 for 5- and 6-ring, respectively. Sabaté et al. (2006) observed low correlations for BaA and CRY, and other HMW PAHs were not either biodegraded nor extracted by HPCD.

Regarding other solubilising agents Latawiec and Reid (2009) used Brij 700 and did not find consistent results across different PAHs in spiked and naturally contaminated soils, but an underestimation was generally observed. Thiele-Bruhn and Brümmer (2004) tested Genapol UDD 88 and SynperonicLF7RA 30 for the extraction of 15 PAHs from 30 industrial soils and found good results (Table 3.5) with a slight overestimation, but the behaviour was similar for all individual groups.

A 3h persulphate oxidation was successfully used to predict the percentage of total PAHs biodegradable (21 days), as well as for 2-4 ring compounds (r^2>0.82), in several historically contaminated soils and sediments (Cuypers et al., 2000). However, for 5 ring PAHs the method could not predict biodegradation, since compounds were better oxidized than biodegraded. Yet, when comparing the residual PAH concentration after biodegradation with the residual concentration after persulfate oxidation, results are much better (Table 3.5). The correlations found by the PAHs group were good in all cases (r^2>0.91) and the slopes were close to one (1.02, 0.99 and 0.91 for 2+3-, 4- and 5+6-ring, respectively). Similar results were obtained by Juhasz et al. (Table 3.5), being the r^2>0.85 for each group, with a slight overestimation (1.3) of PAHs with 5+6 ring plus CRY, but only one soil type was tested. These better correlations observed when comparing residual fractions are likely to be due to the low removal fractions, which give a high uncertainty of results.

Due to the working principles it is not expected that biomimetic methods could be a good predictor of biodegradation. Even so, the single study that has used SPME (Table 3.1), concluded that PHE degradation was significantly related to C_{free} (Table 3.5) up to 144h of biodegradation (being the highest observed for 1-3h). Since the estimated residue fraction of PHE in soil was higher than the measured residue fraction, it's suggested that bacteria are able to degrade PHE in the sorbed form and not just the fraction desorbed.

3.3.2 Bioaccumulation in Earthworms

Earthworms (*Lumbricidae*) are normally used to estimate the potential exposure of soil biota. The major reasons for their usage in standard toxicity tests and what makes them appropriate test organisms are the fact that they live in intimate contact with soil, they uptake contaminants directly from soils (soil solution or ingestion), they show a high degree of pollutant accumulation, and they are of easy handling (Lanno et al., 2004). The main problems concern the pH and other soil property dependence and the limited concentration ranges tolerated for some substances. Ecologically, their importance relies on the fact that they are of extreme importance in terrestrial food chains, they are the largest part of soil biomass (in several soils), and they are essential to the cycling of nutrients, thus having a key role in several soil services (Jager et al., 2000; Ma et al., 1998).

EqPT has been successfully applied to derive ecological screening benchmarks for PAHs in sediment invertebrates; however the applicability of this theory to terrestrial organisms can be questioned. For example, Jager et al. (2003) found that BSAFs obtained in field samples were up to two orders of magnitude lower than the predicted by EqPT and Kreitinger et al. (2007) observed values 3 to 11-folds lower than predicted. Overestimations between 10 and 10,000 of predicted concentration by EqPT were observed for 13 PAHs in worms (Jonker et al., 2007) and between 3 and 11 for 16 PAHs (Kreitinger et al., 2007), both using MGP soils. On the other hand, Krauss et al. (2000) found that EqPT was applicable to predict the bioaccumulation of ∑20PAHs in urban soils.

The deviations to EqPT, in addition to physico-chemical factors as explained previously (section 3.2.2), can be due to the species related differences. Differences between species, which may be related to their behaviour, have been highlighted: residues of PAHs were lower in *Lumbricus rubellus* than in *Eisenia andrei*, on average by a factor of 2, but as high as 17 for BGHI (Jager et al., 2003). One of the assumptions of EqPT is that the uptake of HOCs by organisms occurs via passive diffusion from soil solution through outer membrane. However, earthworms can also access

contaminants from solid phase, through gut uptake, being the importance of this route dependent on the species and the compound. Moreover, Jonker et al. (2007) suggest that compounds can be transferred directly from soil solids to worm tissue by contact between the two phases, however this mechanism is not well studied. It is believed that pore water is the major route of exposure for compounds with log K_{OW}<5, but soil ingestion is very important for more hydrophobic compounds (Ma et al., 1998). Consequently, these authors concluded that EqPT was applicable to predict the bioaccumulation of LMW PAHs in field contaminated soils, but not for the HMW.

Other factors that may cause a deviation in EqPT are biotransformation, active excretion and reproduction. These are mechanism that may be used for depuration of contaminants, explaining differences in metabolic fate of compounds and, consequently, the differences in bioaccumulation rates (Ma et al., 1998; Sijm et al., 2000). For instance, BSAFs were found to be higher for PCBs than for PAHs with similar K_{OW}, probably due to a higher metabolic transformation rate of PAHs (Krauss et al., 2000). Moreover, the elimination rate of PAHs in earthworms decreases with K_{OW}, due to a decrease in metabolic transformation, even though not so as strong as in other HOCs such as chlorobenzenes (Jager et al., 2000; Ma et al., 1998)..

Literature data on earthworm's uptake and accumulation is difficult to compare due to differences in experimental conditions and even presentation of results. Jager et al. (2000) obtained BSAFs for *E. andrei* of 7.3 (kg_{OC}/kg_{lip}) for PHE, 8.2 for FLA, 3.9 for PYR and 2.4 for BaP, in a freshly spiked soil with different concentrations. However, the same authors obtained an average of 0.23 (kg_{OC}/kg_{lip}) (∑14) in field contaminated soils using the same species (Jager et al., 2003). Kreitinger et al. (2007) obtained values ranging from 0.004 (kg_{OC}/kg_{lip}) for NP to 0.227 for BaA in 4 MGP soils using *Eisenia fetida*. Ma et al. (1998) found BSAF values (∑11PAHs) for *L. rubellus* ranging from 0.03 (kg_{OC}/kg_{lip}) to 0.26, with an average of 0.1, in 12 naturally contaminated soils. Parrish et al. (2006) calculated BSAF values for ∑4 PAHs of 0.011 (kg_{OC}/kg_{lip}) for *E. fetida* and 0.007 for *Lumbricus terrestris*, in MGP soils.

Other studies present the % of PAHs accumulated by earthworms and, similarly, a wide range of results were observed. For example, the study of Sun and Li (2005) refers that *E. fetida* accumulated between 0.87 and 3.6% of total PYR in dissimilar soils aged for 120d, whereas Khan et al. (2011), for a similar aging period, presented percentages ranging between 11 and 25% (with higher percentages corresponding to lower contamination levels). Kelsey and Alexander (1997), for a similar aging period, reported 3.3% of PHE accumulated by *E. fetida* and for ANT an uptake percentage of 13.7 after 203d aging was reported by Tang and Alexander (1999). Regarding field

contaminated soils, Bergknut el at (2007) found that *E. fetida* accumulated 0.04% of the total PAHs (∑22) in a MGP soil. Kreitinger et al. (2007) similarly observed an average value 0.06% for the same species, compounds and contamination source. Also in MGP soils, only FLA, PYR, BaA and CRY contained detected levels in earthworms (*E. fetida* and *L. terrestris*) among 12 PAHs analysed (Parrish et al., 2006).

Regarding the effects of soil properties, Sun and Li (2005) observed that percentages of PYR accumulated in earthworms were dependent of soil properties, with a decrease in accumulation following the increase of OM, in spiked soils. In addition, these authors suggest that the clay content may also have some influence on accumulation. Tang et al. (2002) tested different soils and found that the availability of ANT, PYR, CRY and BAP to *E. fetida* varied, being the greatest uptake observed for soil with low content of OM, but any trend was evident for other soils. Jager et al. (2003) stated that OM is not the only property controlling sorption and bioaccumulation. Besides, these authors observed that soils with a higher content of OC and clay, a low pH and low PAHs level, showed higher BSAF values. Hickman and Reid (2005) observed that there were little differences in earthworm (*L. rubellus*) accumulation of PHE between dissimilar spiked soils.

Aging showed an effect on earthworm's accumulation for PHE and PYR (Chung and Alexander, 1999; Sun and Li, 2005), in dissimilar soils. On the other hand, Khan et al. (2011) found that the amount of PYR accumulated by earthworms suffered little changes with aging, for high contamination levels, but great differences were observed in low contamination levels. The effect of concentration has also been studied and, regarding PHE, the assimilated percentage was higher for lower concentrations, although contradictory results were found for PYR (Chung and Alexander, 1999; Khan et al., 2011). However, it should be noted that in low contaminated soils biological factors (e.g.: digestible organic matter) become important (Barthe et al., 2008).

3.3.2.1 Chemical methods for assessing bioavailability to earthworms

Extractions using either pure solvents or mixtures showed significant correlations with earthworm's accumulation, (mainly for 3- and 4- ring PAHs in spiked soils) in all except one study (Table 3.6). The use of 95% EtOH provided good results even when looking to individual compounds (ANT,CRY, PYR, BaP): $r^2 \geq 0.844$ (Tang et al., 2002). Gomez-Eyles et al (2010) also calculated the correlations for the individual PAHs, which were higher than 0.63 using a 50s extraction with pure BuOH. In another study, these authors found similar results for 12 PAHs in industrial soils, for both total concentrations (Table 3.6) or individual compounds (r^2=0.66 for 4 ring PAHs), but in this case 5- and 6-ring PAHs were also tested and correlations were low (0.48).

These authors attempted to improve the relationships by estimating the C_{org} based on EqPT calculations but without success.

Regarding the relationship between the mild solvent extraction and earthworm accumulation, results were not consistent. An underestimation of PHE, PYR, CRY and BAA predictions, in spiked soils, was observed by some authors (Johnson et al., 2002; Kelsey et al., 1997; Liste and Alexander, 2002). However, overpredictions were observed in most studies and, in addition to values presented in Table 3.6, they were also referred in other studies (Gomez-Eyles et al., 2012; Khan et al., 2011; Krauss et al., 2000; Tang et al., 2002). Even though some conclusions are very hard to reach due to the variability of results and methods used, it seems that overestimations are higher when dealing with naturally contaminated samples and that decreases when lowering the percentage of solvent (Bergknut et al., 2007; Gomez-Eyles et al., 2012). However, there is a lack of comparisons between the patterns of PAHs in organism and mild-solvent extractions and few studies tested dissimilar soils, which makes it difficult to arise to conclusions on the method's predictability.

SFE was used to predict availability to earthworms in three studies (Table 3.2): two focused on PHE and PYR availability in dissimilar spiked soils and the other on 16 PAHs present in several MGP soils. In the latter, the prediction of PAHs uptake by *Aporrectodea caliginosa* provided better results (up to a factor of 2.1) when adjusting the EpT for the F_{rap} based on mild SFE, yet no correlations are given (Table 3.6).

SPE extraction with XAD4 was used in only one study (Bogan et al., 2005; Table 3.2) and its authors stated that, for MGP soils with high desorption fractions and uptake by earthworms, the two approaches agreed positively, but not in samples with low to intermediate mobility (based on resin assays). Some authors suggest that a rapid desorbing process assisted with Tenax should be a good predictor of the earthworm uptake, based on findings obtained for benthic organisms in sediment samples (Ten Hulscher et al., 2003). Indeed, Ten Hulscher et al. found significant correlations between concentration accumulated by *L. rubellus* (normalized to the lipid content) and the amount of PAHs (plus chlorobenzenes) desorbed to Tenax (normalized to OC), even though an underestimation of prediction was observed (Table 3.6). Other study also found good correlations for PYR in spiked soils, but in this case its authors related the ln BSAF and desorption percentage (ln), after 12 days (Li et al., 2007). Gomez-Eyles et al. (2010) compared the profile of individual PAHs in Tenax extraction with the worm tissues, observing that the chemical method overestimated bioaccumulation of 2- and 3- ring PAHs whereas 4-ring compounds were underestimated, for both aged and fresh spikes.

Table 3.6 - Comparison between earthworm accumulation and several chemical methods.

Method	Compound	Correlation (r^2)	Relationship	Number of soils; aging[a]	References
BuOH	PHE		0.81±0.15	1, 120d	Kelsey et al., 1997
BuOH	PYR	0.98	-	1, 222d	Khan et al., 2011
BuOH	PYR	0.86	3.477	6, 120d	Sun and Li, 2005
BuOH	PYR, BAA	NS[c]	-	1, 240d	Johnson et al., 2002
BuOH	ANT, FLA, PYR	0.93-0.96[b]	-	1, 203d	Tang and Alexander, 1999
BuOH	∑5 PAHs	0.54	-	1, 6m	Gomez-Eyles et al., 2010
BuOH	∑12 PAHs	0.53	-	10	Gomez-Eyles et al., 2012
MeOH	ANT	0.99[b]	-	1, 203d	Tang and Alexander, 1999
PrOH	ANT, FLA, PYR	0.94-0.99[b]	-	1, 203d	Tang and Alexander, 1999
1% BuOH	∑16	-	3.2	1	Bergknut et al., 2007
50%MeOH	PHE	-	1.52±0.55	1, 120d	Kelsey et al., 1997
50% MeOH	∑16 PAHs	-	125	1	Bergknut et al., 2007
1% MeOH	∑16 PAHs	-	4.0	1	Bergknut et al., 2007
35%EtOH	PHE	-	1.49±0.43	1, 120d	Kelsey et al., 1997
95%EtOH	∑4 PAHs	0.92	-	6, 335d	Tang et al., 2002
SFE	PYR	0.91	5.128	6, 120d	Sun and Li, 2005
SFE	PHE;PYR	0.43-0.45[b]	-	6, 56f	Bielská et al., 2013
SFE	16 PAHs	-	0.8-2.1	3	Kreitinger et al., 2007
Tenax	PYR	0.89	-	6, 120d	Li et al., 2007
Tenax	∑12(+CBs)	0.76	0.28	10	Ten Hulscher et al., 2003
HPCD	PHE	NS[c]	-	4, 37d	Hickman and Reid, 2005
HPCD	PYR	0.98	-	1, 222d	Khan et al., 2011
HPCD	∑5 PAHs	0.51	-	1, 6m	Gomez-Eyles et al., 2010
HPCD	∑12 PAHs	0.10	-	10	Gomez-Eyles et al., 2012
HPCD	∑16 PAHs	-	24.8	1	Bergknut et al., 2007
SPME	∑12 PAHs	0.46	-	10	Gomez-Eyles et al., 2012
SPME	∑16 PAHs	-	1.2	1	Bergknut et al., 2007
POM-SPE	∑12 PAHs	0.46	10	10	Gomez-Eyles et al., 2012
C18	∑4 PAHS	0.87	-	6, 335d	Tang et al., 2002
C18	15 PAHs	0.47-0.87	0.49- 4.4	25	Krauss and Wilcke, 2001
SPMD	∑16 PAHs	-	2.4	1	Bergknut et al., 2007
TECAM	NP;PHE; PYR;BAA	0.97-0.93	0.033-0.61	10, 150d	Tao et al., 2008b
TECAM	NP;PHE; PYR;BAP	0.59-0.82	0.84-1.22	18	Tao et al., 2009

[a]Number of soils with dissimilar properties tested and maximum aging period in days (d) or months (m) for spiked soil; [b]correlation coefficients (r); [c]Not significant

In spite of the study conducted by Khan et al (2011) that found a very good correlation between HPCD extractions and earthworm accumulation for PYR, the other studies indicate poor or no correlation (Table 3.6). Moreover, HPCD extractions systematically overpredicted earthworm accumulation in spiked and naturally contaminated soils (Bergknut et al., 2007;

Gomez-Eyles et al., 2012; Khan et al., 2011). Gomez-Eyles et al. (2012) estimated C_{org} based on EqPT calculations but results were not improved (either correlations or relationship). Tween-80 was tested by Bergknut et al. (2007) and, considering this surfactant a lipophilic phase, it underpredicted the earthworm lipid normalized concentration by a factor of 0.64.

Some authors concluded that the prediction using non-exhaustive extractions was only slightly improved when compared with total extractions, especially if comparing compound profiles. In general non-exhaustive methods show higher percentages of LMW PAHs, whereas the percentage of HMW PAHs in total extractions is higher, being closer to what is observed in earthworms. This is most likely to be due to mechanisms of biotransformation or the importance of other routes of uptake rather than soil solution, as explained before.

Theoretically, the application of biomimetic methods, with or without the use of EqPT calculations, would be a best approach to predict PAH accumulation in earthworms. Despite the fewer studies conducted, comparing with non-exhaustive extractions, these were mostly performed in dissimilar naturally contaminated samples (Table 3.2). For example, Gomez-Eyles et al. (2012) used SPME and POM-SPE to predict the bioavailability of 12 PAHs in naturally contaminated soils, and in spite of low correlations found for total PAHs (Table 3.6), they were better when looking to each group, with the exception of HMW PAHs. The r^2 was 0.67 for 3-ring, 0.8 for 4-ring and 0.31 for 5+6-ring PAHs in the case of SPME and of 0.51 for 3-ring, 0.7 for 4-ring and 0.2 for 5+6-ring PAHs, in the case of POM-SPE. This study, along with the one conducted by Jonker et al. (2007), that also tested heterogeneous sets of naturally contaminated soils, concluded that either SPME or POM-SPE predicted bioaccumulation by a factor of 10, i.e both under- or over-predicted within this factor. However, these authors suggest that this could be a promising method, since current methods (total extractions using or not EqPT model) overpredict accumulation by a factor of 10-10,000. It is also suggested that the estimation of C_{org} could be improved by an accurate determination of BCF for key species instead of assuming that BCF values are equal to K_{OW} (Jonker et al., 2007).

Krauss and Wilcke (2001) used C18 disks and derived a model (for individual 20 PAHs) based on log K_{disk} and log K_{OW} values which could predict the BSAFs for dissimilar urban soils (Table 3.6). When directly comparing K_{disks} with BSAF values, the latter were underestimated for HMW PAHs, corroborating the hypothesis of the existence of other uptake routes such as gut. The other study that used C18 (Table 3.6) also showed good correlations for individual compounds ($r^2 \geq 0.770$), but an underestimation was observed for all compounds, being higher for BAP.

SPMD was only used in one study and one tested soil, giving a slight overestimation (Table 3.2 and Table 3.6). TECAMs were tested in two studies, and good correlations were generally observed (Table 3.2 and Table 3.6). In a first study, Tao et al. (2008b), performed a direct comparison between the concentration in TECAM and in the earthworm (without lipid normalization) and observed that the slope of linear relationship decreased with compound hydrophobicity being as low as 0.033 for BAP (Table 3.6). According to the authors, the general underestimation may be due to differences in exposure routes and activity of earthworms in soil (e.g. ingestion and excretion of contaminants, or due to disturbance of soils caused by earthworms). However, in a further study using field contaminated soils (Tao et al., 2009) a relationship of about 1:1 (Table 3.6) was found, although in this case a lipid normalization was performed.

3.3.3 Bioaccumulation Assays using Plants

Root uptake of HOCs by plants is an important pathway for their transfer into the food chain (Bogolte et al., 2007; Gao and Collins, 2009). Moreover, it is important to understand the availability of these compounds to plants in order to predict the potential for phytoremediation (Ahn et al., 2005). Direct relationship between PAH concentration in soil and plants has been observed, suggesting a pathway from soil to roots. As for the other organisms, this uptake will depend on the plant species, contaminant type and soil properties.

Root uptake has been shown to be a passive and diffusive process and it is known that the composition of plant root tissues, particularly its lipid content, controls the uptake of HOCs. For this reason, the lipid content of a plant is normally included in plant uptake models (Gao and Collins, 2009; Zhang and Zhu, 2009). EqPT calculations have been applied to predict concentration in plants (C_{plant}), assuming that uptake occurs mainly through pore water. Hence, the estimation of C_{plant} can be carried out as described for other organisms (C_{plant}=RCF*C_{free}), where RFC is the root concentration factor. As for BCF, it has been shown that the relationship between the log RFC and log K_{OW} is linear (Gao and Collins, 2009). However, other studies suggest that assuming RFC, specifically lipids-water partition coefficients (K_{lip}) equal to K_{OW}, may underestimate the K_{lip}, especially for more hydrophobic compounds (Zhang and Zhu, 2009). These authors suggest that both plant lipids and carbohydrates regulate PAH uptake and the affinity of PAHs for lipids is 1.64 orders of magnitude higher than for carbohydrates. Nevertheless, roots of species such as ryegrass contain 98 times more carbohydrates than lipids and, therefore, this process should be considered. As a result of these recent findings, improvements on EqPT calculations have been

suggested (Gomez-Eyles et al., 2012). These authors suggest the separate calculation of C_{plant} for these two major root components: lipids and carbohydrates (ch): $C_{plant,lip}=C_{free}K_{lip}$; $C_{plant,ch}= C_{free}K_{ch}$. However, this approach requires knowing the species specific relationships between K_{lip} and K_{ch} with K_{OW}.

Other important processes involved in the uptake of contaminants by plants are the sorption of PAHs to the cell wall and the production of root exudates (Ling et al., 2009; Zhang and Zhu, 2009). Exudates, such as low molecular weight acids, are capable of disrupting the sequestering soil matrix and as a consequently increase the availability of PAHs. On the other hand, exudates may increase the microbial community and therefore the biodegradation process (Ling et al., 2009).

Differences in uptake between species were observed by Tang et al. (1998), for instance, which reported a % of ANT uptake of 0.62% by wheat and 0.35% by barley after a 200d aging. As for other organisms differences between compound uptake have been observed. For example, in MGP soils, 2 plant species were tested and the % dissipation after one year was between 1.5 for indeno(1,2,3-cd)pyrene and almost 90% for 3- and 4-ring PAHs (Cofield et al., 2008). In a coke oven site soil, where profiles were dominated by HMW PAHs no significant differences in the total PAHs concentration were observed after the third year of phytoremediation (Ahn et al., 2005). On the other hand, in agricultural soils the uptake by wheat roots showed little differences between the four PAHs analysed: 0.16% (for PHE) and 0.32% (for BaP) (Tao et al., 2008a).

3.3.3.1 Chemical methods for assessing phytoavailability

In general good linear relationships have been found between mild solvent extractions and accumulation by different plant species in spiked soils (Table 3.7). Yet, the study of Gomez-Eyles et al. found lower correlations for individual compounds (r^2>0.57) than for the total, which wasn't significant for PHE. Tao et al. (2008a) also stated that good linear relationships were observed for field contaminated samples, but no values were provided. On the other hand, the study of Gomez-Eyles et al (2012), also conducted in naturally contaminated samples, showed very low correlations, being similar even when looking for individual groups. Even when accumulation data was correlated with the concentrations predicted by using EqPT calculations the r^2 were similar, except for the 4-ring group which rises to 0.54. Regarding the relationship between the two approaches, it was observed that, for a 2 month aging period BuOH extractions underestimated the accumulation of 2- and 3-ring PAHs and overestimated for 4-ring PAHs (Gomez-Eyles et al., 2010). On other studies, very high overestimation factors were found either for individual PAHs

(2-, 3-, 4- and 5-ring) or for the ∑12 compounds naturally contaminated soils (Table 3.7). Using 50%MeOH, lower overestimation factors were recorded, in comparison with those obtained with BuOH, decreasing with the increase of MW.

Cofield et al. (2008) concluded that a 24h Tenax extraction was a predictor of the labile fraction of LMW changing during phytoremediation, especially for 3-ring (Table 3.7). Yet, for the 4-ring PAHs there is an underestimation of availability (lability) and for the 5 ring compounds no trends were observed. Gomez-Eyles et al. (2010) compared the profiles of Tenax extractions and plant tissues, and in spite of a significant difference between PAHs profiles (slightly underestimated for 3- and 4-ring and overestimated for 2-ring PAHs) they were closer than the observed values for earthworms.

Gomez-Eyles et al. (2010) state that HPCD could be a good predictor of accumulation of PAHs in ryegrass roots for total PAHs (Table 3.7), despite the correlation not being significant for PHE when considering individual compounds. This method seems to underestimate concentrations of 2- and 3-ring and overestimate 4-ring PAHs in root tissues after an aging period of 2 months. In another study using industrial soils, these authors concluded that HPCD extractions largely overpredict the plant accumulation in naturally contaminated soils (Table 3.7), and very low correlations were observed (the highest r^2=0.43 for 4-ring PAHs). Similarly to BuOH extractions, correlating accumulation data with the concentrations predicted by EqPT does not improve the relationships.

Gomez-Eyles et al (2012) studied the ability of SPME fibers to predict the accumulation in plants, and found that measurements were closer to the 1:1 line than other methods. However, correlations were very low even when looking at individual groups (ranging from 0.07 for 3-ring PAHs to 0.25 for 5+6-ring PAHs). Similar results were observed using POM-SPE, with correlations for individual groups ranging from 0.13 for 5+6-ring PAHs to 0.27 for 4- ring PAHs. Moreover, a trend to underestimate accumulation using both methods was observed, which could be due to both: a) the presence of soil particles in roots or b) the production of exudates by soil roots.

Lipid-containing passive samplers such as TECAMs are expected to be a good surrogate of plant uptake, since it is passive and diffuse processes and root lipids have an important role on the uptake of HOCs. Yet, only one study used these membranes to predict root uptake (Table 3.3).

Concentrations in TECAMs were 40 times higher than in roots, and although these values become closer after lipid normalization, they were still high (maximum of 7.1). The authors suggested that relating the amounts of PAHs uptaken by TECAMs to the amounts uptaken by roots provides better results (Table 3.7). Gomez-Eyles et al. (2012) suggested that the better

correlation obtained by TECAM, may be because these samplers are buried close to the plant roots.

Table 3.7- Comparison between plant accumulation and several chemical methods.

Method	Compound	Correlation (r2)	Relationship	Number of soils; aging[a]	References
BuOH	ANT	0.89-0.93	-	1, 203d	Tang and Alexander, 1999
BuOH	NP;PHE; PYR;BAP	-	163-277	15	Tao et al., 2008a
BuOH	∑5 PAHs	0.93	-	1, 6m	Gomez-Eyles et al., 2010
BuOH	∑12 PAHs	0.38	10-1000	10	Gomez-Eyles et al., 2012
MeOH	ANT	0.93-0.94	-	1, 203d	Tang and Alexander, 1999
PrOH	ANT	0.92-0.95	-	1, 203d	Tang and Alexander, 1999
50%MeOH	NP;PHE; PYR;BAP	-	3.6-38	15	Tao et al., 2008a
Tenax	∑5 PAHs	0.92	0.633	1	Cofield et al., 2008
HPCD	∑5 PAHs	0.97	-	1, 6m	Gomez-Eyles et al., 2010
HPCD	∑12 PAHs	0.26	10-1000	10	Gomez-Eyles et al., 2012
SPME	∑12 PAHs	0.27	-	10	Gomez-Eyles et al., 2012
POM	∑12 PAHs	0.16	-	10	Gomez-Eyles et al., 2012
TECAM	NP;PHE; PYR;BAP	0.80-0.93	0.82-1.25	15	Tao et al., 2008a

[a]Number of soils with dissimilar properties tested and maximum aging period in days (d) or months (m) for spiked soil

3.3.4 Ecotoxicological Assays/Molecular Biology Methods

Toxicity testing, with whole soils and ecologically relevant soil organisms, indirectly measures (through several different endpoints) the biological availability of contaminants in soils. The advantages are both the fact that they only respond to the bioavailable fraction of contaminants and also that they reflect the site-specific effects of contaminants (mixtures and metabolites). However, the non-selectivity of these tests may also be a drawback, especially important in urban soils, for instance, due to the presence of multiple contaminants. In ideal conditions a chemical-specific test should be used concomitantly to identify the contaminants of potential concern present in the different environmental matrices or test substrates. Recently, the use of metabolomics has been suggested, which may distinguish between organic and inorganic contaminant exposure (Brown et al., 2010). This method identifies the metabolic responses of earthworms to a sub-lethal exposure using ^{1}H nuclear magnetic resonance (NMR) (Brown et al., 2010). Genotoxic assays using solid-phase, aqueous or solvent extracts of soil have been also used to assess the effect of organic contamination in soils (Alexander and Alexander, 2000).

Table 3.4 presents an overview of existing studies comparing ecotoxicological assays with chemical assays. Alexander and Alexander (2000) used BuOH extraction and a bacterial

genotoxicity assay for testing soils spiked with BAP, with good results (r=0.691). These authors also observed that, excluding soils with <0.7% OC, significant negative correlations were found between bioavailability, %OC and CEC, but not with clay or surface area. Kreitinger et al. (2007) observed that a mild SFE extraction (normalized to OC) was related to acute toxicity of ∑16 PAHs to *E. fetida* in 16 industrial soils. Similarly, Čvančarová et al. (2013) concluded that the F_{rap}, estimated by sequential SFE, correlated with the toxicity to *E. fetida*. Yet, results of the other toxicity tests were unreliable due to the presence of potentially toxic elements present in soil samples. Cofield et al (2008) concluded that a 24h Tenax has the potential to predict the toxicity response of several bioassays (nematode and earthworm survival, and lettuce emergence) to the ∑5 PAHs in a MGP soil, with an r^2 between 0.8 and 0.94, with exception of microbial respiration (r^2=0). Brown et al. (2010) used ^{1}H NMR metabolomics to monitor *E. fetida* responses to PHE and compared results with total and HPCD-extracted (r^2=0.64, slope=1.2) concentrations. The authors did not found any differences between the two extraction methods but, only one freshly spiked soil was used.

The use of passive samplers to estimate C_{free} has been proposed to complement toxicity tests, since these tests are often performed at high concentrations and the saturation of aqueous phase may occur, which could lead to underestimation of results (ter Laak et al., 2006a). Styrishave et al. (2008) found a strong correlation (value not shown) between toxicity of PYR to the springtail *Folsomia candida* (generation of reproductive effect concentrations, EC_{50}) and estimated pore water concentrations in spiked soils. Jonker et al. (2007) observed that using C_{free} determined by SPME allowed to correctly predicted *E. fetida* mortality (∑13 PAHs in MGP soils) in 87% of the cases. The approach consisted in converting predicted individual PAH concentration in worm lipids to concentrations on a molar basis (mmol/kg lip). Further, concentrations were summed and compared to critical body residues from literature (between 50 and 200 mmol/kg lip).

3.4 PROBLEMS AND CHALLENGES FACED BY CHEMICAL METHODS FOR ASSESSING AVAILABILITY

The determination of biodegradation or bioaccumulation does not necessarily measure the bioavailability of contaminants but rather the integration of complex interactions that occur. Therefore, even when significant correlations between available fractions assessed by chemical methods and bioavailability are found, the findings usually do not provide a 1:1 relationship. There are several factors that may affect this relationship, being the most important ones the biotransformation or metabolism of HOCs (effects on bioaccumulation measures) and the access to contaminants through other routes of exposure rather than aqueous phase.

In the case of biodegradation, since contaminants are biodegraded one can assume that depletive methods (bioaccessibility) would be a more reliable approach. Yet, if desorption is rate-limiting these methods, especially the ones based on the measurement of the F_{rap}, will underestimate biodegradation. Moreover, for species able to produce surfactants or that can access the sorbed phase, chemical methods based on uptake from aqueous phase will also tend to underestimate the availability. Therefore, the use of solubilising agents such HPCD provides more reliable prediction as shown by several studies.

Based on EqPT, it is expected that, for higher organisms, such as plants or earthworms, biomimetic methods could be feasible (e.g. TECAMS for plants and SPME for earthworms). However, the application of this theory to terrestrial organisms has been questioned. Moreover, for earthworms, which may have complex accumulation mechanisms or may access contaminants from both solid and aqueous phase, the determination of the contaminants in soil solution may not be the most appropriate estimation of bioavailability. If substances are biotransformed it is likely that chemical methods that measure pore water concentration will overestimate. On the other hand, if organisms have other routes of exposure, they will underestimate. In addition, the estimation of bioconcentration factors based on K_{OW} when applying EqPT models may not be adequate. The solution could be the inclusion, in the prediction models, not only of specific properties of the contaminants (e.g. lipids-water partition coefficients - K_{lip}), but also organism's specific uptake and detoxification mechanisms for key species.

Other problems are related to the complexity of soil matrix, such as the mechanisms of sorption/desorption and heterogeneity. A high variability of results (for both bioassays and chemical methods) has been observed for different soils. Moreover, many studies were conducted in spiked soils, which may not simulate real contaminated samples, especially due to the quality of OM, sources and aging. For example, it is known that PAHs in naturally contaminated soils such as MGP sites are recalcitrant and the presence of soot or BC can seriously affect contaminant desorption. Therefore, in addition to physico-chemical properties of soils and compounds, the origin of contamination may play a role in chemical and biological availability. In order to overcome the resulting overestimation given when using generic K_{OC} on EqPT models, the use of site-specific values has been suggested (Gomez-Eyles et al., 2012).

The behaviour of individual PAHs is very different and they should be treated separately or at least by ring size. However, some studies do not consider PAHs individually or by groups, but rather its sum. In addition, little is known for HMW PAHs, mainly because most of spiked samples target only LMW PAHs, and normally they are strongly sorbed in field contaminated samples,

showing in general lower chemical availability. Yet, it shouldn't be ignored that bacteria communities could be unable to degrade these compounds. On the other hand, for higher organisms, other routes of exposure (rather than pore water) and lower biotransformation can have a stronger influence on bioaccumulation rates of HMW PAHs. For example, Bergknut et al. (2007) observed that PAHs composition in earthworms was different from that of the chemical methods (either non-exhaustive extraction or biomimetic methods), but similar to the soil (total extraction), i.e. with a higher percentage of HMW PAHs. This has important implications on a risk assessment basis, since HMW PAHs are carcinogenic and mutagenic and the effects of the long term exposition to soils contaminated with HMW PAHs are still not clear.

In order to include chemical availability in risk assessment it is necessary to have consistent results (reproducibility and good correlations) for different types of soils, but not necessarily a 1:1 relationship. A systematic overprediction can be seen as the worst-case scenario and ideally a prediction factor should be obtained (for individual or groups of compounds and for a target species) as a function of soil properties. In order to understand and to compare results a standardization of methods, especially for bioaccessibility which is operational defined is needed. Moreover, little attention has also been given to the uncertainty of analytical measurements, especially when dealing with very low concentrations.

It's very likely that it will not be possible to replace bioassays by chemical methods to assess availability, at least on a short time basis. However, the importance of chemical methods is beyond the potential replacement of bioassays. Chemical availability can help to understand the bioavailability process and the behaviour of PAHs in soils, being a useful basis for producing descriptive models. Bioaccessibility may be more important to demonstrate the virtual absence of bioavailability when contaminants are strongly sorbed (Reichenberg and Mayer, 2006). Moreover, the potentially available concentrations are much more conservative, despite being more realistic than the current approach. Nevertheless, in spite of the overestimation of risks on a short term, F_{rap} could become available under changing conditions (Brand et al., 2013).

Data about chemical availability can be used as a screening tool (with or instead of total extractions) since they can be a faster and initial approach to estimate availability. Chemical methods can be also used in combination with ecotoxicological tests, to find out which are the contaminants responsible for a given toxicity data. More specifically, Loibner et al. (2006) suggest two different approaches for the inclusion of chemical availability data in risk assessment. The first is the use of bioaccessible data in the refined screening phase (Tier 2), by directly comparing the concentrations obtained in the non-exhaustive extractions with soil screening levels. This

approach is based on the fact that soil screening levels are obtained using freshly spiked soils and therefore no aging is likely to occur. However, to use this direct comparison it is necessary that the non-exhaustive method used extracts around 100% of the freshly spiked concentrations. The second approach involves the use of biomimetic methods in a higher tier (Tier 3) and compares the results with the water quality objectives. Yet, this approach should be held with care and the uncertainty should be taken into account since the sensitivity of aquatic and terrestrial species may not be comparable. Moreover, the applicability of aquatic bioassays to assess soil ecotoxicity has been discussed (Antunes et al., 2010).

Brand et al. (2013) suggest the inclusion of both type of methods (non-exhaustive and biomimetic) in the Tier 3 of the risk assessment, using a similar approach to the one described by Loibner et al. (2006). Measuring chemical availability is suggested in samples that show concentrations higher than intervention values, which further directly relates the freely available concentration with risk levels for water, or the bioaccessible fraction with risk levels in soil.

Chapter 4

LEVELS, SOURCES AND POTENTIAL RISKS OF ORGANIC POLLUTANTS IN URBAN SOILS[1]

[1] Adapted from:

- Cachada A, Pato P, Rocha-Santos T, da Silva EF, Duarte AC. Levels, sources and potential human health risks of organic pollutants in urban soils. Sci Total Environ 2012a; 430: 184-192.

- Cachada A, Dias A, Pato P, Mieiro C, Rocha-Santos T, Pereira M, et al. Major inputs and mobility of potentially toxic elements contamination in urban areas. Environ Monit Assess 2013; 185: 279-294.

4.1 INTRODUCTION

PAHs and PCBs are primarily emitted to the atmosphere, and after their transport over short and long distances, in both gaseous and particulate forms, they accumulate in soils after dry and wet atmospheric deposition (Heywood et al., 2006; Motelay-Massei et al., 2004; Ravindra et al., 2008). Therefore, even that hydrophobic organic contaminants (HOCs) are present in soils all over the world, urban areas are normally enriched in these contaminants due to the proximity to both diffuse or point sources. As referred before, due to their physico-chemical characteristics, these contaminants are likely to be retained in soils for many years having negative impacts on ecosystems and on human health (CCME, 2010; USEPA, 2011b).

Considering the risk assessment framework presented in Chapter 2, the first step to assess the potential hazard risk of these compounds is to measure their levels in the environment. In addition, the identification of contaminant sources is also a critical step in HOCs risk assessment and management, especially in complex environments such as urban areas, in which there is not a single source but several point and diffuse sources. For this reason, multivariate statistics or simple compounds profiles and isomer ratios are normally used for source identification, even with some drawbacks (Cachada et al., 2009; Ravindra et al., 2008; Yunker et al., 2002). Specifically, for PAHs these approaches have been used to provide information on the possible origin (petrogenic versus pyrogenic origin) and sources of contamination (e.g.: tire debris, biomass or fuel combustion). The relationship between HOC contamination and potentially toxic elements (PTEs) can also provide indications on their sources. Some studies reported that HOC and PTE contamination can be related (Cachada et al., 2012b; Maliszewska-Kordybach et al., 2009), but most studies on urban soil quality do not take into account both types of contaminants (Jiang et al., 2011; Peng et al., 2011a; Wu et al., 2011). Soil properties are also often excluded from urban soil studies and as a consequence their role on HOCs fate in soils is not fully understood.

Despite the lower ecological sensitivity of urban soils when compared with natural areas, they are responsible for several ecosystem services being very important to characterize the risks associated to their contamination. Therefore, some of the models presented in section 2.4 (TU and HQ calculations) were used to identify potential risks to the environment caused by HOCs in soils of the studied areas. This deterministic approach has been considered adequate for identification of possible impacts and for prioritisation in a first tier of risk assessment (RA) (SCCS et al., 2012). Due to the proximity of urban residents with soils, it becomes also very important to assess their potential risks to human health. Thus, in the present study, the evaluation of the potential risks that HOCs pose to human health was made by applying the methodologies

described in section 2.5. Therefore, the non-cancer risks as well as cancer incidence and the mutagenic risks resulting from the exposure to urban soils during daily and recreational activities were calculated. Despite the several limitations (e.g. methodology may not reflect the Portuguese reality; low number of samples; high associated uncertainty) of this approach, it is an indication of the potential risks.

This chapter intends to be a comprehensive study on selected HOCs in Lisbon and Viseu urban soils, assessing the levels of these contaminants and potential risks to environment and human health, identifying sources and studying their behaviour. The relationship between HOCs (PAHs and PCBs) and between HOCs and PTE contamination was assessed, as well as the factors affecting their distribution (soil properties).

4.2 MATERIAL AND METHODS

4.2.1 Sampling and soil characterization

Fifty one composite samples (0-10 cm) were collected from Lisbon urban area and fourteen from Viseu, considering different land uses (ornamental gardens, parks, open spaces and roadsides). Sampling location maps (Figure 4.1) were produced with ArcGis® Software (version 9.3).

For a general characterisation of soils and potentially toxic elements (PTEs) analysis, soils were collected by using a plastic spade previously cleaned with distilled water and ethanol and transferred into plastic bags. Once at the laboratory the samples were dried (oven dried at 40°C until constant weight), sieved (<2 mm) and grounded (<180 µm, using an agate mill), according to ISO 11464 method. For organic contaminants analysis samples were collected by using a metal spade (previously cleaned with distilled water and acetone) and stored involved in aluminium foil. Samples were then air dried in a cold and dark place, sieved to <2 mm using a stainless steel sieve, homogenised and frozen (-20°C) wrapped in aluminium foil. Soil water content was determined by weigh loss after drying the soil samples in an oven (105°C overnight or until constant weight).

The general characterization of soils included: the pH (in $CaCl_2$ and water; ISO 10390:1994); the organic matter content (LOI, 16h at 430°C) (Schumacher, 2002); cation exchange capacity (CEC) (ISO 13536:1995); elemental C, N and H analysis (microanalyser CNHS-932 (LECO); particle size distribution (sand, silt and clay fractions quantified using a Sedigraph 5100 Micromeritics®). In addition, organic carbon (OC) content of Lisbon soils was also determined by elemental analysis (Skalar Primac SCN), after elimination of carbonates with H_3PO_4.

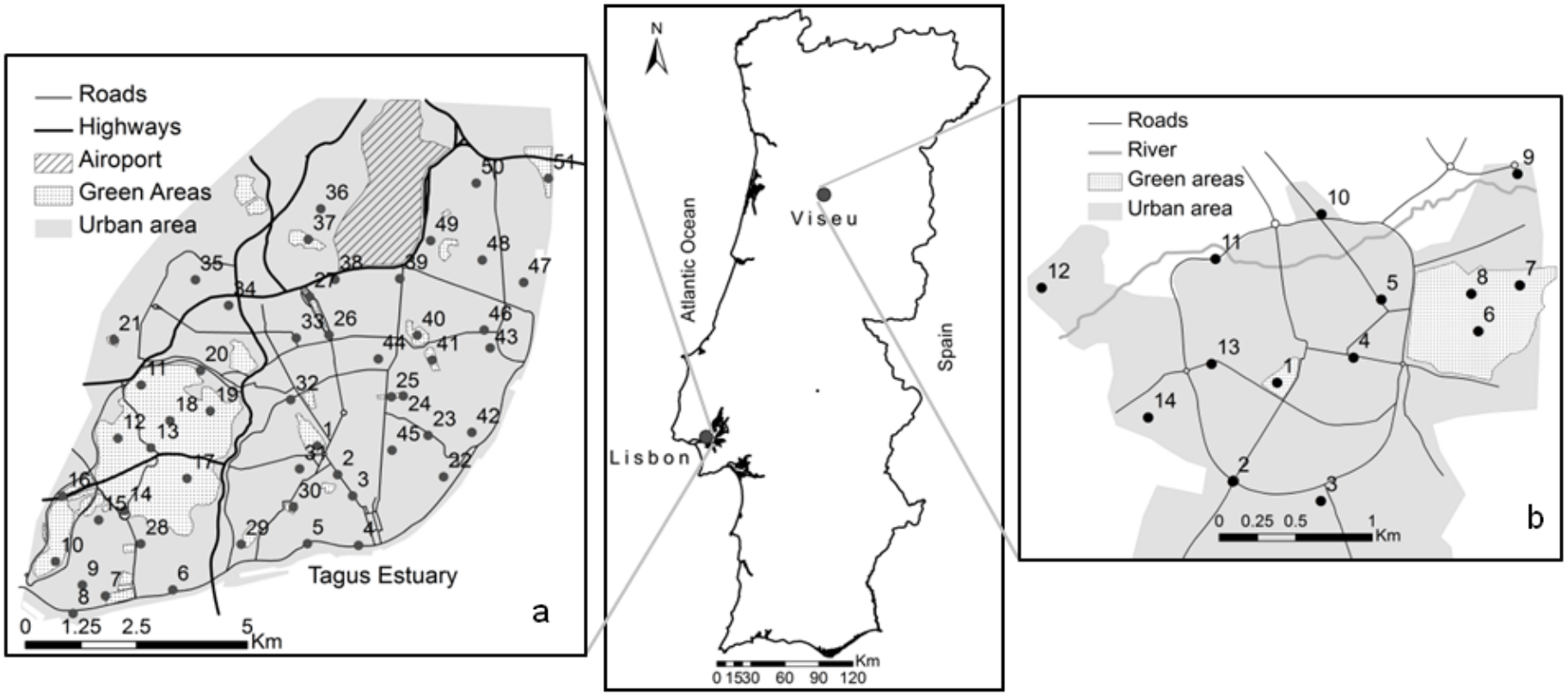

Figure 4.1 - Location of the cities and of the sampling sites in Lisbon (a) and Viseu (b).

The pseudo-total content of 33 PTEs (Ag, Al, As, Ba, Bi, Ca, Cd, Co, Cr, Cu, Fe, Ga, K, La, Mg, Mn, Mo, Na, Ni, P, Pb, S, Sb, Sc, Se, Sr, Th, Tl, Ti, U, V, W and Zn) were determined by ICP-MS (Perkin Elmer Elan 6000/9000) in an accredited laboratory (AcmeLabs, Canada). Samples (0.5 g of the fraction <0.18 mm) were digested with Aqua Regia at 95°C, for 60 min. Total Hg concentrations were determined by thermal decomposition atomic absorption spectrometry with gold amalgamation using AMA-254 (LECO model AMA-254), directly on dried soil samples.

4.2.2 Methodology for the determination of PAHs and PCBs

For extraction of PAHs and PCBs, 10 g of soil was Soxhlet extracted with 90 mL of hexane/acetone (2:1) for 8 h, at a rate of 10 cycles h^{-1}, in a prewashed glass fibre thimble (USEPA, 1996). The extracts were concentrated using a rotary evaporator (at 30°C) and submitted to a clean-up using solid phase extraction (SPE) cartridges filled with 1g of silica (Supelclean® LC-Si), 2 g of neutral alumina (Supelclean® LC-AL-N), both 3% deactivated, and 0.5 g of Na_2SO_4 at the top. The elution was made with 20 mL of hexane/DCM (9:1) and 10 mL of hexane/DCM (2:1). The eluate was then concentrated down to 2 mL using a rotary evaporator, transferred to a vial and the volume was then reduced to approximately 0.2 mL under a gentle stream of pure nitrogen, after the addition of isooctane. For analysis of PCBs, the extracts were re-dissolved in 2 mL of hexane and further underwent a clean-up with 2 g of acidic silica (30% concentrated H_2SO_4, w/w), eluted with 15 mL of hexane and the solvent changed to isooctane before analysis.

Extracts were analysed using gas chromatography with mass spectrometry detection (GC/MS-QP5050A, Shimadzu Corporation), using helium as a carrier gas and a SPB-5 (5% diphenyl, 95% dimethyl polysiloxane) fused silica capillary column. The detection was achieved with a mass selective detector using electron ionization (EI) in the single ion monitoring mode (SIM). The injection volume was 1 µL and the splitless injection mode was used. The carrier gas flow was 1 mL/min for PAHs and 0.7 mL min^{-1} for PCBs. For the analysis of the 16 PAHs the injector temperature was 290°C and the interface temperature was 300°C. The oven temperature was programmed as follows: 40°C for 2 min, increase at a rate of 10°C min^{-1} until 220°C, followed by 6°C min^{-1} until 260°C, and finally at 3°C min^{-1} until 300°C and kept for 6 min. For PCBs the injector temperature was 280°C and the interface temperature was 300°C. The oven temperature was programmed as follows: 40°C for 2 min, increase at a rate of 10°C min^{-1} until 290°C and kept for 8 min.

4.2.3 Quality assurance and quality control (QA/QC) procedures

QA/QC procedures included replicates, procedure blanks and the analysis of certified reference materials. Replicate analysis of the soil samples gave an uncertainty <20% for each individual organic contaminant. Method blanks were used in every tenth sample in order to detect possible interferences from the reagents, glassware and other processing hardware, and results were always below the instrumental detection limit. Detection limits ranged from 0.15 to 0.63 μg kg^{-1} for PAHs and from 0.02 to 0.14 μg kg^{-1} for PCBs, depending on the specific compound. Recoveries of certified reference materials ranged from 90±4 to 100±2 % for PCBs (LGC 6113) and from 78±3 to 99±4 % for PAHs (RTC-CRM124-100). Internal standards (decachlorobiphenyl for PCBs and a deuterated-PAHs mixture for PAH determination) were added to samples before extraction and recoveries ranged from 60 to 109%.

4.2.4 Statistical methods

Univariate statistical methods were initially performed to check the variability of the data and the presence of anomalies. Normality was tested (Shapiro-Wilk's test for normality, skewness and kurtosis values) and boxplots were also obtained. Since some parameters did not follow a normal distribution, Spearman correlations were used to test the relationships between variables.

In cases where the compounds were present below the detection limit, missing values were considered as equal to one tenth of the detection limit. This value was chosen in order to minimize an overestimation of samples with many PCB congeners below the detection limit. This is a relevant issue in the present study because levels found in some of the samples are low and very close to the detection limit.

Cluster analysis (CA) was performed in order to classify similar observations into groups, following an agglomerative hierarchical clustering method (Ward method with squared Euclidean distances). CA included data on general parameters (pH, OC and clay particle size), since the parent material is a very important factor that will influence the natural concentrations and the soil properties. Since these statistical methods are severely affected by the data distribution and the presence of outliers, a log transformation was performed. Furthermore, the data was standardized to avoid the effects resulting from differences in units of measure.

The 95% percentile of the upper confidence on the mean (UCL), the UCL (95%), used for human health risk assessment analysis was calculated non-parametrically using the statistical package ProUCL® available by USEPA. Other descriptive and multivariate statistical analyses (Spearman correlations, CA) were performed in SPSS®.

4.3 RESULTS AND DISCUSSION

4.3.1 Characterization of soils from the two urban areas

Table 4.1 shows the results obtained for the general parameters of soils collected in the cities of Lisbon and Viseu. The high median values of pH and total carbon (TC), observed in Lisbon are in line with the calcareous nature of these soils. The highest organic matter content (OM), cation exchange capacity (CEC) and clay particle size contents were found in samples located at south-west (mainly samples 7, 9-14, 17-21; Figure 4.1a), reflecting the presence of chromic vertisols (heavy texture), which dominate this area. On the other hand, highest percentages of sand size particles observed in the rest of the area points toward the sandy nature of these soils. Hence, the high variability of results observed for general parameters seems to be primarily related to the type of soils found. Even so, the introduction of soils from elsewhere can be a contributing factor to the variability of results observed.

Table 4.1 - Results of the general parameters determined in Lisbon and Viseu: pH, organic carbon (OC), organic matter (OM), cation exchange capacity (CEC) and particle size (sand, silt and clay).

Parameter	Lisbon				Viseu			
	Mean	Median	Min	Max	Mean	Median	Min	Max
pH ($CaCl_2$)	7.2	7.3	6.5	7.6	5.3	5.3	3.9	6.9
TC (%)	4.1	4.1	0.94	8.6	2.2	1.5	0.67	7.4
OC (%)	2.6	2.5	1.0	6.0	-	-	-	-
OM (%)	7.2	6.4	1.6	19	5.4	4.2	2.9	17
CEC (cmol kg^{-1})	20	19	3.9	52	11	9.5	5.1	26
Sand (%)	56	57	11	96	64	62	42	86
Silt (%)	29	29	2.9	47	30	31	11	44
Clay (%)	15	13	1.0	44	6.2	5.0	2.8	14

The results of general parameters in Viseu soils are also in agreement with the nature of the parent material and the type of soil of the region. Soils from Viseu city are moderately acid, with medium pH values, low TC content, low OM and low CEC (Table 4.1). The particle size results showed lower variability when compared with the Lisbon results, showing also higher median percentages of sand and silt size particles.

Results of PTEs (Table 4.2 and Table 1 of Annex III) were discussed in a previous work (Cachada et al., 2013) and, since this is not the focus of the present study, only a brief summary is given. As for general parameters, the pseudo-total concentrations of PTEs seem to reflect the geological

background of each city. Lisbon soils show very high concentrations of elements that occur in basaltic or calcareous rocks such as Ca, Co, Cr, Ni and Sr, whereas Viseu soils are enriched in elements which are typical of schist and granitic rocks such as Al, Ga, K, La, Sc, Th, Ti and U. After applying Cluster Analysis it was possible to group PTEs according to their possible sources (Cachada et al., 2013). The origin of the high levels of Co, Cr and Ni in Lisbon soils was identified as telluric (basalts from the Lisbon Vulcanic Complex). For As, low levels were observed (similar to background), with exception of few hotspots with no specific source addressed. Other elements (Cu, Pb, Zn and Hg) showed relatively high concentrations (when comparing with other cities and with background) due to anthropogenic sources (mainly traffic and industry). In Viseu, Co, Cr and Ni showed low concentrations, being also related with local geology. On the other hand, As showed very high concentrations all across the city, despite its telluric origin. Also for the other elements, with exception of a hotspot identified in the city centre (sample 1), the high median values observed (in some cases similar to Lisbon) should be due to geologic reasons.

Table 4.2 - Descriptive statistics of PTEs concentrations in Lisbon and Viseu urban soils.

Element	City	Mean	Median	Min	Max	RSD (%)
As (mg kg^{-1})	Lisbon	5.3	4.4	0.50	29	87
	Viseu	30	24	17	58	50
Co (mg kg^{-1})	Lisbon	13	6.8	0.6	49	100
	Viseu	5.9	5.9	3.1	7.9	22
Cr (mg kg^{-1})	Lisbon	38	16	1.0	172	116
	Viseu	11	10	6.0	17	25
Cu (mg kg^{-1})	Lisbon	37	29	3.5	143	70
	Viseu	33	27	6.1	78	61
Ni (mg kg^{-1})	Lisbon	43	20	2.0	209	121
	Viseu	5.0	4.5	3.3	9.7	42
Pb (mg kg^{-1})	Lisbon	89	62	4.8	561	110
	Viseu	106	46	13	817	195
Zn (mg kg^{-1})	Lisbon	97	88	7.0	269	55
	Viseu	88	80	47	190	43
Hg (mg kg^{-1})	Lisbon	0.36	0.18	0.01	3.8	169
	Viseu	0.26	0.11	0.02	1.6	158

4.3.2 Levels and sources of PAHs in urban areas

Concentrations of the ∑16PAHs obtained in surface soils of both cities are presented in Figure 4.2a and Table 4.3. The accumulation of PAHs in urban soils over many years and the presence of more

sources explain the higher concentrations measured in larger and industrialized cities like Lisbon in contrast to Viseu (around 5 times lower median than in Lisbon). Levels found in Lisbon soils are also much higher than the ones found in another small but industrialized Portuguese city, Estarreja (Table 4.3). On the other hand, the mean concentration and maximum values observed in Lisbon were comparable or even higher than the ones observed in other studies (with the exception of Glasgow) which reflects the presence of important hotspots, as observed in Figure 4.2a. When comparing median concentrations with cities from all over the world (Table 4.3), Lisbon soils showed lower levels than other European cities, but generally higher than the ones found in Asian cities. Higher concentrations in European urban areas were observed by other authors (Liu et al., 2010a; Wilcke, 2007; Zhang et al., 2007) and a similar trend was also observed for global background soils (Nam et al., 2009), in which climate was the main reason pointed out for this difference.

The fairly short urbanization history of the city of Viseu, the recent growth in population, the size of its urban area and the lack of major industrial activity (with subsequently less fossil fuel combustion), explain the low concentrations measured. For example, Estarreja, which is a very small Portuguese town, presents slightly higher contamination levels of PAHs due to the presence of industry and nearby busy roads (Cachada et al., 2012b). Median concentration found in Viseu was lower than that in Swiss soils (163 μg kg^{-1}), unpolluted soils from Spain (242 μg kg^{-1}), natural and agricultural soils (<250 μg kg^{-1}) from Italy and Poland, or to the background (136 μg kg^{-1}) referred for Europe (Fabietti et al., 2010; Maliszewska-Kordybach et al., 2009; Desaules et al., 2008; Nadal et al., 2007; Nam et al., 2009).

In Lisbon soils, high PAH concentrations (Figure 4.2a) were observed in historical parks and gardens essentially located in the city centre (samples 5, 7, 8, 23, 31 and 45; Figure 4.1a). This area is close to the river and it is the oldest part of the city, having a high population density. In Viseu, the highest concentration observed (Figure 4.2a) was also in a historical park at the city centre (sample 1; Figure 4.1b). Historical sites are especially important when tracing urban diffuse contamination, as a result of long-term exposure to contaminants leading to an accumulation in soil's upper layer (Liu et al., 2010a; Zhang et al., 2007). As a result, these soils are usually multi-contaminated as observed in both cities studied, since some of the outlier values observed for PAHs coincide with the ones of PCBs (Figure 4.2b) and PTEs (samples 5, 7, 31 in Lisbon and sample 1 in Viseu) (Cachada et al., 2013).

In the past, the oldest area of Lisbon was very industrialized, but nowadays mobile sources such as roadway traffic, aircraft and shipping are probably the most important sources. Samples

5, 7 and 8 (Figure 4.1a) were all collected very close to a busy road and a railway line; another outlier value was observed near the airport and an important highway (sample 38). However, other important PAHs sources should be considered (Ravindra et al., 2008): samples 7 and 8 are also from sites located nearby the docks; sample 5, in addition to being collected in a site close to the most important dock, it was also close to a shipyard activities; sample 23 was obtained nearby a crematorium; and sample 38 was very close to the only incinerator of hospital hazard waste in Portugal.

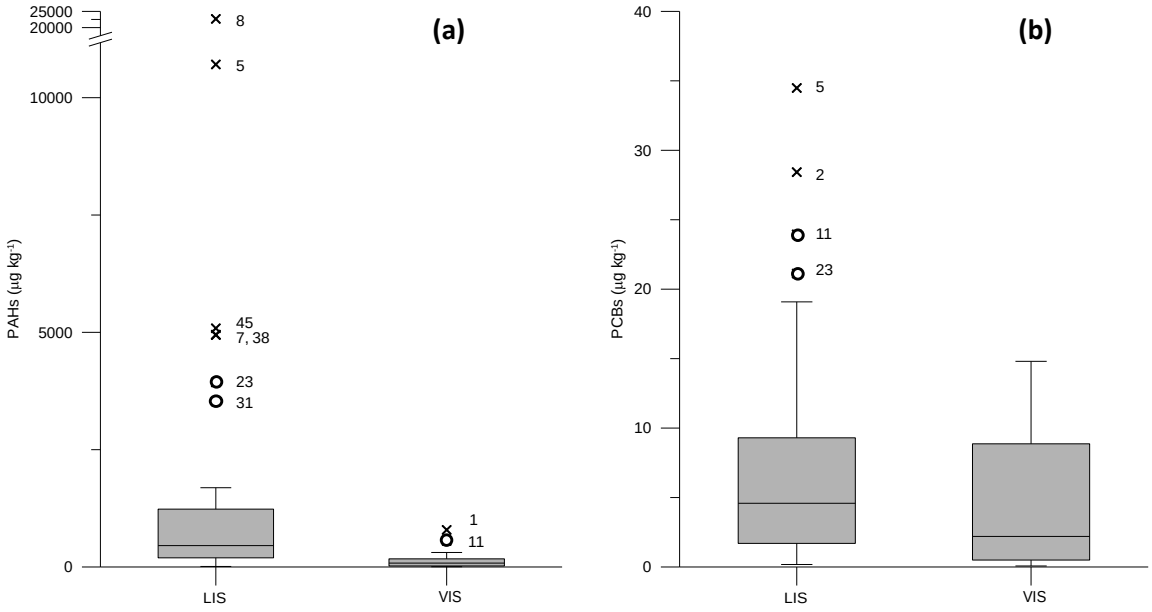

Figure 4.2 - Boxplots showing the variation of PAHs (a) and PCBs (b) concentrations in Lisbon (LIS) and Viseu (VIS). Boxes define the interquartile range and the line is the median. Outliers are defined as values between 1.5 and 3 box lengths (○) and extreme values as more than 3 box lengths (x).

In Viseu, none specific point source could be identified. The major sources of PAHs in Viseu are, in fact, likely to be diffuse sources such as wood and coal combustion (domestic heating, nearby agriculture, forest fires) and diesel engines from domestic heating and from the nearby highway, which is the most important in the country regarding freight. The great contribution of low temperature sources in Viseu compared to Lisbon, in which there is a great input of high temperature industrial combustion, seems to be reflected in the PAH profiles, since Viseu shows a slightly higher median of percentages of low molecular weight (LMW) PAHs (Figure 4.3 and Figure 1 of Annex III) (Boonyatumanond et al., 2007; Ravindra et al., 2008; Wilcke, 2007).

Table 4.3 - Median, mean concentration and range of HOCs (μg kg^{-1}) in urban soils around the world.

City	Nº inhab.	∑PAHs				∑PCBs				Reference
		Median	Mean	MIn	Max	Median	Mean	Min	Max	
Lisbon, Portugal	564,567	456	1,544	6.3	22,670	4.6[b]; 2.3[c]	7.0[b]; 4.1[c]	0.18[b]; 0.06[c]	34[b]; 18[c]	This study
Viseu, Portugal	47,250	83	169	6.0	790	2.2[b]; 0.76[c]	4.6[b]; 1.7[c]	0.08[b]; 0.08[c]	15[b]; 6.8[c]	This study
Estarreja, Portugal	7,000	98	-	27	2,016	8.8[d]	-	2.3[d]	55[d]	Cachada et al., 2012b
Aveiro, Portugal	73,500	-	-	-	-	7.9[d]; 2.6[e]	-	0.62[d]; 0.15	73[d]; 41	Cachada et al., 2009
Glasgow, Scotland	600,000	8,337[a]	11,930[a]	1,487[a]	51,822[a]	22[d]; 9.4[e]	-	4.5[d]; 1.9[e]	78[d]; 43[e]	Cachada et al., 2009; Morillo et al., 2007
Torino, Italy	900,000	704[a]	1,990[a]	148[a]	23,500[a]	14[d]; 6.6[e]	-	1.8[d]; 0.72[e]	172[d]; 86[e]	Cachada et al., 2009; Morillo et al., 2007
Ljubljana, Slovenia	300,000	791[a]	989[a]	218[a]	4,488[a]	6.8[d]; 2.1[e]	-	2.8[d]; 0.67[e]	48[d]; 29[e]	Cachada et al., 2009; Morillo et al., 2007
Uppsala, Sweden	136,500	-	-	-	-	5.7[d]; 2.3[e]	-	2.3[d]; 0.54[e]	77[d]; 47[e]	Cachada et al., 2009
Terragona, Spain	155,000	-	438	42	1472	-	4.4[c]	0.19[c]	10[c]	Nadal et al., 2007
Beijing, China	18000,000	688	1,228	93	13,141	13[f]; 3.2[g]	12[f]; 3.1[g]	nd[f;g]	37[f]; 9.3[g]	Peng et al., 2011a Wu et al., 2011
Shangai, China	20600,000	314	1,700	62	31,900	-	3.1[h]	0.23[h]	11[h]	Liu et al., 2010bb; Jiang et al., 2011
Hong Kong	6800,000	140	-	ND	19,500	3.9[c]	4.8[c]	1.6[c]	9.9[c]	Chung et al. 2007; Zhang et al., 2007
Harbin, China	3800,000	301	837	202	3,256	2.1[i]; 0.47[c]	2.2[i];0.53[c]	0.53[i]; 0.19[c]	6.2[i]; 1.2[c]	Ma et al., 2009

[a]∑15PAHs; [b]∑21PCBs; [c]∑7indicatorsPCBs; [d]∑19PCBs; [e]∑5indicatorsPCBs;[f]∑18PCBs; [g]∑6indicatorsPCBs; [h]∑74PCBs; [i]∑44PCBs; nd = not detected

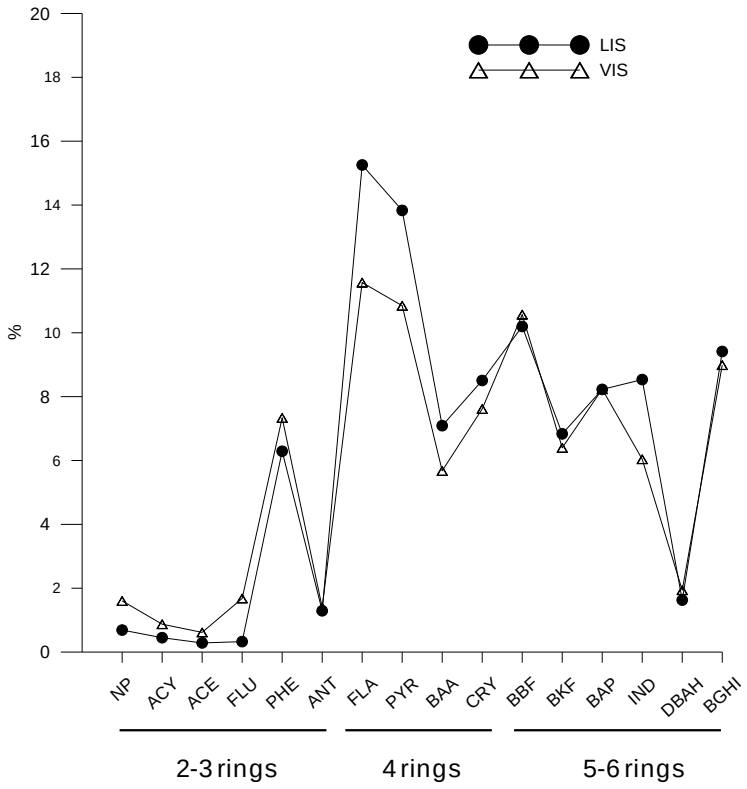

Figure 4.3 - Median percentage of individual compounds of PAHs.

These LMW compounds are normally present in gaseous form and they are related with ubiquitous atmospherically dispersed emissions, being subjected to longer distance transport (Wilcke, 2007). Climate may, therefore, play a role in this difference of profiles, since the higher levels of precipitation and lower temperatures observed in Viseu may enhance wet deposition process and condensation, respectively (Desaules et al., 2008). On the other hand the higher temperatures observed in Lisbon can strongly affect the degradation and volatilisation of LMW PAHs (Nadal et al., 2004).

The high molecular weight (HMW) PAHs (≥4 rings) are dominant in the two cities studied (Figure 4.3 and Figure 1 of Annex III). This predominance is typical in urban areas and may be due to (i) the higher persistence of these compounds in soils, (ii) the predominance of combustion over petrogenic sources, and (iii) the tendency of HMW PAHs to accumulate in soils that are close to emission sources (Chung et al., 2007; Liu et al., 2010b; Morillo et al., 2007). The most abundant PAHs found in both cities, and especially in Lisbon, were fluoranthene (FLA) and pyrene (PYR) (Figure 4.3), compounds that are usually associated with fossil fuel (especially diesel), coal and biomass combustion, but also with tire debris (Boonyatumanond et al., 2007; Ravindra et al.,

2008). The high contribution of benzo(ghi)perylene (BGHI) is related with vehicle emissions (especially light-duty), and the greater contribution of indeno(1,2,3-cd)pyrene (IND) in Lisbon can be associated, in addition to traffic, with oil combustion or industrial sources (Boonyatumanond et al., 2007; Oliveira et al., 2011; Ravindra et al., 2008). Yet, using these composition patterns to trace the sources in urban areas is very difficult due to the presence of mixed sources and because PAHs composition profiles can be weathered (Brandli et al., 2008; Katsoyiannis et al., 2011; Zhang et al., 2005). The use of isomer ratios of selected pairs of isomers (Table 2 of Annex III) has been widely applied in order to overcome these constrains. However, it was not possible to address specific sources in the present study, since different ratios pointed to different results as observed by other studies (Brandli et al., 2008; Katsoyiannis et al., 2011). Even when applying the correction factors for soils indicated by Zhang et al. (2005), as suggested in the study of Brandli et al. (2008), the conclusions remain similar.

4.3.3 Levels and sources of PCBs in urban areas

Concentrations of the ∑21PCBs obtained in surface soils of both cities are presented in Figure 4.2b. Table 4.3 shows, in addition to the results for ∑21PCBs, the sum of concentrations of the 7 indicators (∑7indicatorsPCBs). The variability of results found in the present study is very high, with some samples showing relatively high concentrations (even in Viseu), as observed in other cities around the world (Table 4.3). The concentrations observed in the Lisbon urban area were not very high, and comparable to the ones found in other smaller cities like Ljubljana, Uppsala and Tarragona. In bigger and very industrialized cities, like Torino and Glasgow, much higher concentrations were observed. However, levels from Lisbon were similar or even higher than the ones found in Asian cities (Table 4.3), and the reason should be the same given for PAHs. Median concentration of PCBs in Lisbon was lower than in Aveiro and Estarreja soils, which are much smaller Portuguese cities, but likely to be affected by a nearby chemical complex and a pulp and paper mill (Cachada et al., 2009; Cachada et al., 2012b).

Mean concentration of PCBs in Viseu is somewhat high, only slightly lower than Lisbon's value. Concentrations of PCBs in Viseu were higher than those measured in unpolluted soils from Spain (∑7PCBs with a mean of 0.77 μg kg^{-1}), close to the values in natural Italian and British soils (∑32PCBs with a mean of 3.5 μg kg^{-1} and 5.0 μg kg^{-1}, respectively) but lower than those found in Switzerland soils (∑7PCBs with a median of 1.6 μg kg^{-1}) (Desaules et al., 2008; Fabietti et al., 2010; Heywood et al., 2006; Nadal et al., 2007).

Only Lisbon showed outlier values (Figure 4.1b), three of them in the city centre and within those, two are historical sites (5 and 23), as observed for PAHs. In addition, sample 5 was collected very close to the shipyard activities where PCBs were widely used (e.g. paints, coatings), and sample 23 is from a site located near a crematorium (PCBs may be released due to their presence in materials, precursors and chlorine in the cadavers and in some co-combusted plastics). Other specific hotspots may be due to the introduction of contaminated foreign soil or due to the use of contaminated sewage sludge as a fertilizer, such as the case of sample 2, which is from a recent flowerbed in the city centre. Accidental spill or improper waste disposal may also be a source of PCBs, as the case of sample 11 since this sample was from a forest area inside a park and no other specific source could be addressed. Sample 38, despite not being considered an outlier, is very important since it shows high PCBs concentration (19 μg kg^{-1}) and it was collected close to the incinerator of hospital waste, which may be a source of PCBs).

In Viseu, high PCBs concentrations were observed in sample 6, from a forest area inside a park and therefore the reason could be an accidental spill or improper waste disposal; and in sample 13, which is from a recent residential area, in which new soil is likely to have been introduced. Since no other specific sources are likely to be present, the most probable origin of PCBs in Viseu soils is atmospheric transport from other contaminated areas. The slightly higher contribution of lower chlorinated PCBs (≤4Cl) in Viseu when compared to Lisbon (Figure 4.4 and Figure 2 of Annex III), may not only be a consequence of long-range atmospheric transport but also of climate differences, as referred for PAHs (Motelay-Massei et al., 2004; Zhang et al., 2007). Nevertheless, lower-chlorinated PCBs are present in lower percentages than the higher chlorinated ones (>4Cl), especially in Lisbon (Figure 4.4).

In both cities the most abundant congeners were PCB 138, 153 and 180 (Figure 4.4), and similar to results observed in other European cities, in British soils and in unpolluted soils from Spain (Cachada et al., 2009; Heywood et al., 2006; Nadal et al., 2007). Overall, the profiles from both cities were similar to those observed in other cities, including other Portuguese cities, where it was found that the profiles resemble Arachlor 1260 (Cachada et al., 2009; Cachada et al., 2012b). Similar findings can be addressed for the present study, especially for Viseu, yet Lisbon PCB profiles also show some similarity with Arachlor 1254 (Figure 4.4). Moreover, the high contribution of high chlorinated PCBs (HC-PCBs) in this city can also be attributed to the presence of nearby high temperature sources (e.g. incineration).

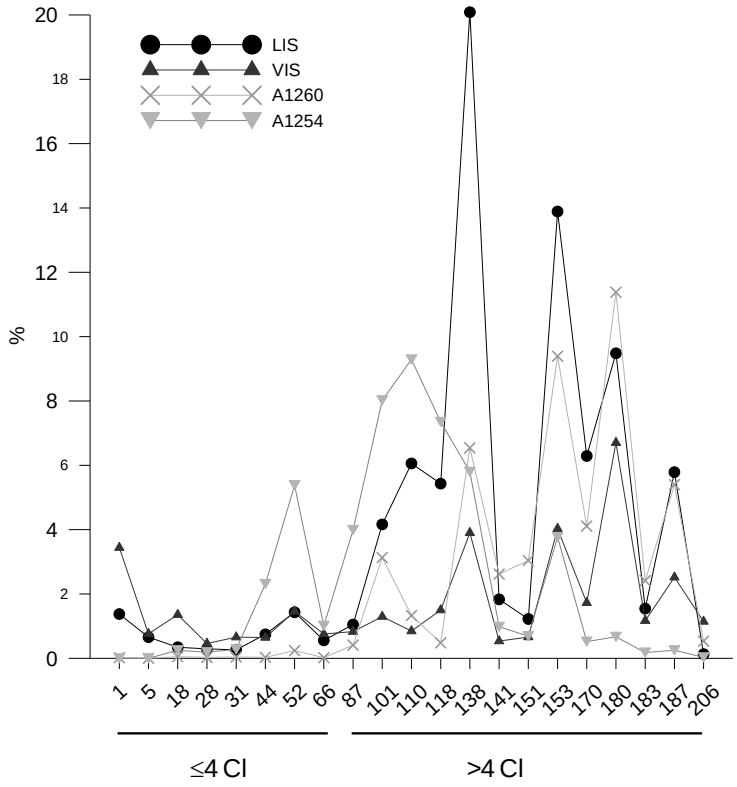

Figure 4.4 - Median percentage of individual compounds of PCBs observed for the two cities. The percentage of compounds present in Arachlor 1260 (A1260) and Arachlor 1254 (A1254) are also shown.

4.3.4 Controlling factors and relationship among contaminants

The dendrogram of cluster analysis (CA) was similar in both cities, showing three major groups (Figure 4.5). In both cases it was observed a group formed by PTEs which included the elements considered from telluric origin (Co, Cr and Ni) (Cachada et al., 2013). Another group, also observed in both cities, is formed by HOCs, anthropogenic PTEs (Cu, Pb, Zn and Hg in Lisbon; Pb and Hg in Viseu) and OC (Figure 4.5). Therefore, this cluster can be related to a common anthropogenic source or may be governed by the HOC relationships, since these elements are normally strongly associated with soil organic matter. In order to investigate whether the relationships observed between HOCs and anthropogenic PTEs were due to common source or if they might be affected by soil properties, the data were normalized for the OC content. The CA remains similar after data normalization for both cities (data not shown), however some correlations changed indicating that OC may affect the relationships observed (Tables 3 and 4 of Annex III).

After normalization, PAHs were still positively correlated with PCBs in Lisbon soils (Table 4 of Annex III), although this correlation was stronger for the HC-PCBs (ρ=0.57, $p<0.01$). Similarly, correlations between PTEs and PCBs were low (Table 4 of Annex III), but stronger when looking only for HC-PCBs (≥0.41, $p<0.05$). Since the presence of HC-PCBs can be attributed to the presence of nearby high temperature sources, its relationship with PAHs and PTEs may indicate the presence of common sources such as industry or incineration, as observed in other studies conducted in impacted areas (Cachada et al., 2012b). In Viseu, no correlations were observed after normalization between PCBs and other contaminants, indicating distinct sources of these compounds.

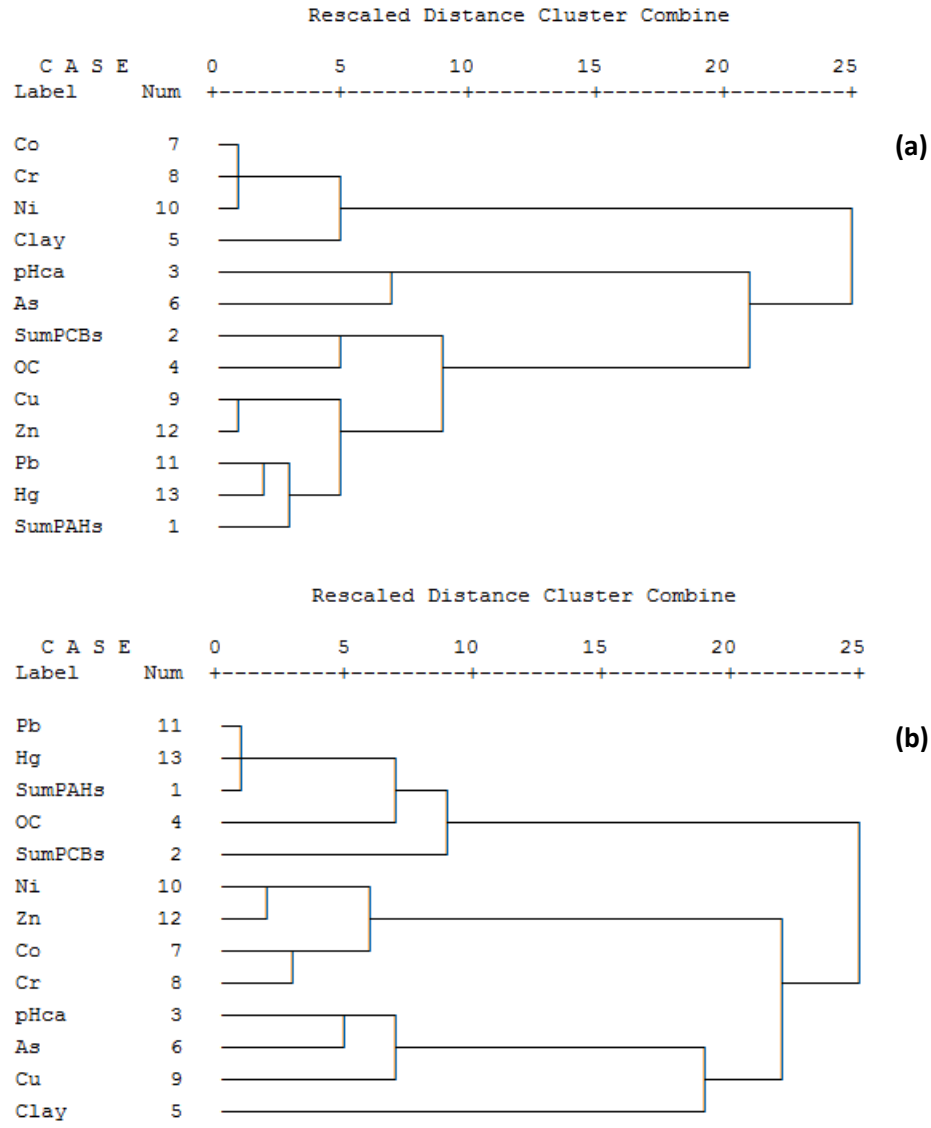

Figure 4.5 - Dendrogram of the cluster analysis of HOCs, PTEs and general parameters for Lisbon (a) and Viseu (b) soils.

PAHs have been found to coexist with PTEs due to similar sources, such as motor vehicle emissions (Cachada et al., 2012b; Maliszewska-Kordybach et al., 2009). The present study confirms this association, especially between PAHs, Pb and Hg in both cities, with correlations higher than 0.77 ($p<0.01$; Table 4 of Annex III) even after OC normalization. In addition, significant correlations ($p<0.01$; Table 4 of Annex III) were also observed for the other two urban elements in Lisbon soils (Cu, Zn), suggesting common sources, such as traffic.

In both cities correlations with OC were similar for LMW and HMW PAHs, although slightly higher for the second group. Stronger correlations between HMW PAHs and OC had been already observed, especially in more contaminated areas, and they can be related with their lower mobility and the fact that they are normally associated with particles and, therefore, tend to deposit near the sources (Brandli et al., 2008; Maliszewska-Kordybach et al., 2009).

For PCBs, a significant correlation with OC was observed for HC-PCBs ($\rho=0.62$, $p<0.01$) in Lisbon soils. Similar observations were found in British soils (Heywood et al., 2006), in Swiss soils (Brandli et al., 2008) and in urban soils from Torino (Cachada et al. 2009). These positive correlations between OC and soil PCBs have been addressed to the concept of "hopping" (compounds that undergo a series of emission-deposition cycles that lead to accumulation in soils enriched in OC) (Heywood et al., 2006). Even though it is generally accepted that OC is a sorbent of PCBs (Ma et al., 2009; Zhang et al., 2007), many studies did not found any relation as observed for Viseu (Cachada et al., 2009; Jiang et al., 2009; Liu et al., 2010b).

Correlations were stronger between organic carbon and PCBs than with PAHs in Lisbon soils, reflecting the stronger binding of the first group to soils as observed in other studies (Desaules et al., 2008; Heywood et al., 2006). However, different results were observed in Viseu. This ambiguity has been already reported (Desaules et al., 2008; Zhang et al., 2007) highlighting that the fate of HOCs in the environment may be highly variable and that there is a lack of understanding of controlling factors, especially in urban soils.

4.3.5 Identification of potential risks of HOCs in urban areas

As a first approach to assess potential problems concerning contamination of Lisbon and Viseu soils with PAHs and PCBs, levels were compared with generic guidelines. Twenty percent of Lisbon samples can be considered slightly contaminated since they presented levels above the target value from the Dutch guidelines (Table 2.4), but only two samples were considered not suitable for residential land use and none needs urgent remediation. Regarding precaution levels from German (Table 2.5), 10% of samples exceeded this value for BAP and ∑16PAHs, indicating a

certain chance of future soil problems. Comparing with the Finish thresholds the values were exceeded in: 14% for BAP; 4% for FLA; 2% for ∑16PAHs, BkF and BAA. According to these guidelines a site-specific risk assessment should be carried out in these areas. No Lisbon sample showed concentrations above the generic guideline values established for PCBs. All samples from Viseu were below the generic guideline values for both PAHs and PCBs. Therefore, according to this first screening only levels of PAHs in Lisbon area can be a potential concern.

As a second approach, major risks to the environment (ERA) and human health (HHRA) were individually identified. Therefore the methodologies presented in sections 2.4 (ERA) and 2.5 (HHRA) were applied.

4.3.5.1 Potential risks to the environment health (ERA)

As a first screening of environmental risk assessment (ERA) the toxic units (TU) were calculated for each compound (Table 4.4) as shown in Eq. 2.2, i.e., the ratio between soil concentration and the predicted no effect concentration (PNEC). The PNEC values used are presented in Table 2.1 and they were derived by applying an assessment factor to the lowest available NOEC value, given by the EU risk assessment report for coal-tar pitch (EU, 2008). Since values were derived for a standard soil with 2% OC and 3.4% OM, they were first corrected for the properties of the soils tested. In Lisbon, risks were always below 1 for the 2 and 3-ring compounds (NP, ACY, ACE, FLU, PHE and ANT) and for FLA. For all other compounds at least one sample showed a ratio above 1, and for BAA and BAP in more than 10% of samples unacceptable effects on organisms are likely to occur. In Viseu, none of the samples showed risks above 1. The toxic units of the mixture (TUm) were also calculated, assuming the concentration addition model, and results can also be found in Table 4.4. The median value of Lisbon is higher than 1 and it can reach values as high as 50. Moreover, in 55% of Lisbon soils sampled unacceptable effects on organisms are likely to occur as a result of the exposure to the mixture of PAHs. In Viseu the median was lower than 1 and only two samples showed a value for the TUm above the unit.

The hazard quotients (HQs), i.e. the ratio between soil concentration and the soil screening levels, were then calculated (Eq. 2.1) using the different guidelines for protection of environmental health, and the minimum, median and maximum values obtained for Lisbon soils are presented Table 4.5. In the case of Finish and Spanish guidelines, values were corrected for the properties of the soils tested. Comparing levels of PAHs observed in Lisbon soils with the quality criteria recommended in the Danish guidelines presented in Table 2.6, which were based on ecotoxicological effects, it was observed that 14% of samples exceeded the guideline values for

∑5PAHs (HQ>1) and 25% for BAP. On the other hand none of the samples showed levels above the Finish lower value neither for the serious risk contamination (SRC) value from Netherlands shown in Table 2.6 (results not shown). Considering the latter guidelines, only the SRC values were used since the maximum permissible concentration (MPC) values presented in the table are comparable to the PNEC value (used to calculate TU as described previously). Comparing with Spanish guidelines for protection of soil organisms shown in Table 2.7, only FLA and BAP exceeded the recommended levels in 8 and 10% of samples, respectively. The results indicate that unacceptable effects on organisms are likely to occur in these samples and a site-specific RA should be conducted. PAHs levels from Lisbon soils were also compared with the Canadian soil quality guidelines for environmental health (SQG_E), or with the interim SQG values when SQG_E are not available, for residential soils presented in Table 2.8. Only in one sample adverse effects of biological importance are likely to occur as a result to the exposure to BAA, BBF, BKF and IND.

In Viseu no sample showed levels above the guidelines presented for both PAHs and PCBs. Five Lisbon samples showed a HQ above 1 when comparing with the Denmark ecotoxicological quality criteria for PCBs, being the highest value 1.8.

Table 4.4 – Minimum, median and maximum values of toxic units (TU) for individual compounds and the toxic units of the mixture (TUm). The percentage of samples with TU and TUm above 1 is also shown.

	Lisbon				**Viseu**		
	Min	Med	Max	TU>1 (%)	Min	Med	Max
NP	0.00	0.00	0.05	0	0.00	0.00	0.02
ACY	0.00	0.00	0.24	0	0.00	0.01	0.08
ACE	0.00	0.02	0.24	0	0.00	0.00	0.01
FLU	0.00	0.00	0.01	0	0.00	0.00	0.03
PHE	0.00	0.01	0.21	0	0.00	0.01	0.10
ANT	0.00	0.03	0.83	0	0.00	0.00	0.11
FLA	0.00	0.03	1.0	0	0.00	0.01	0.07
PYR	0.00	0.04	1.5	2	0.00	0.03	0.56
BAA	0.00	0.25	7.6	12	0.00	0.01	0.07
CRY	0.00	0.04	1.4	2	0.00	0.02	0.22
BBF	0.00	0.09	3.6	4	0.00	0.01	0.15
BKF	0.00	0.05	2.8	4	0.01	0.10	1.06
BAP	0.00	0.40	17	20	0.00	0.02	0.24
IND	0.00	0.17	6.0	8	0.00	0.01	0.26
DBAH	0.00	0.07	3.3	6	0.00	0.03	0.28
BGHI	0.00	0.14	4.5	8	0.00	0.00	0.00
TUm	0.03	1.45	50	55	0.03	0.27	3.1

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Table 4.5 - Minimum, median and maximum value for the hazard quotient (HQ) and Hazard Index (HI) of Lisbon urban soils, according to different soil screening guidelines.

	Denmark			**Finland**			**Spanish**			**Canada**		
	min	med	max	min	med	max	min	med	max	min	med	max
NP	-	-	-	0.00	0.00	0.03	0.00	0.02	0.50	0.00	0.01	0.20
FLU	-	-	-	-	-	-	0.00	0.00	0.21	-	-	-
PHE	-	-	-	0.00	0.01	0.51		-	-	0.00	0.01	0.19
ANT	-	-	-	0.00	0.00	0.09	-	-	-	0.00	0.00	0.10
FLA	-	-	-	0.00	0.02	0.89	0.00	0.12	4.45	0.00	0.00	0.07
PYR	-	-	-	-	-	-	-	-	-	0.00	0.01	0.35
BAA	-	-	-	0.00	0.01	0.35	0.00	0.01	0.16	0.00	0.03	1.46
BBF	-	-	-	-	-	-			-	0.00	0.04	2.44
BKF	-	-	-	0.00	0.01	0.44			-	0.00	0.03	1.82
BAP	0.00	0.36	22	-	-	-	0.00	0.14	5.96	0.00	0.00	0.11
IND	-	-	-	-	-	-	-	-	-	0.00	0.04	1.90
DBAH	-	-	-	-	-	-	-	-	-	0.00	0.01	0.44
∑PAHs	0.00	0.22	12	0.00	0.03	0.92		-	-			
HI	-	-	-	0.00	0.07	2.0	0.00	0.32	11	0.00	0.17	9.1

The hazard index (HI), i.e. the sum of individual HQ, for the different PAHs guidelines was also calculated (Table 4.5), with the maximum value ranging from 2 to 11 in Lisbon soils, but the median value was always below one, indicating that only a small fraction of samples can be a potential hazard: 4% of samples when considering the Finish guidelines, 16% for Spanish and 8% for Canadian. In the case of Viseu the HI using Finish values showed a maximum of 0.13, whereas for Spanish it was 0.68 and for Canada it was 0.31. Again, Viseu soils showed levels of PAHs that can be considered safe.

4.3.5.2 Potential risks to the human health (HHRA)

The risk-based soil criterion for protection of human health from Canada, based on PAH carcinogenic effect (CCME, 2010), indicates a safe level of 600 µg BAPeq kg^{-1} considering a total lifetime cancer risk (TLCR) of 10^{-6} (Table 2.9). Only 4 samples from Lisbon showed concentrations above this value, being the mean value for the ∑BAPeq in Lisbon of 229 µg BAPeq kg^{-1} and in Viseu of 24 µg BAPeq kg^{-1} (Table 5 of Annex III).

Taking into consideration the concentrations recommended in the Danish guidelines for BAP, DBAH and ∑5PAHs(Table 2.9), they were exceeded in 27%, 10% and 14% of samples, respectively, indicating that in these areas exposure should be reduced. In addition, two samples were above the cut off value for BAP, indicating that remediation could be necessary. Comparing with Dutch, Spanish, Italian and the USEPA screening levels for residential soils (Table 2.9 and Table 2.11), levels of PAHs in Lisbon soils were always exceeded (especially for BAP), but always in less than 30% of samples. Viseu did not present samples with PAHs levels above the selected guidelines, and PCBs were always detected at safe levels in both cities.

Non-cancer risks were calculated for a residential exposure, using the equations presented in section 2.5.1 and the USEPA default calculation parameters presented in Table 2.2, being the HQ was always below 1. However, this was not the case for carcinogenic compounds, as expected from the previous comparison with the safe levels. The rationale for the calculation of carcinogenic risks was presented in section 2.5.2 and it were used the USEPA default calculation parameters presented in Table 2.2. For residential and occupational exposure, risks were calculated for all sites, since they are all public areas and either inside or nearby residential areas. Risks associated with recreational sites were calculated only including parks and gardens, in which recreational activities are likely to occur (2h/day and 26d/year). The exposure risks were calculated for different point estimates: mean, median, minimum, maximum concentrations, and for the 95% percentile of the upper confidence level of the mean (UCL 95%). The UCL (95%), along

with the assumed exposure parameters, is considered a reasonable maximum exposure, according to USEPA (2011b). For PAHs the risks were calculated based on the sum of BAPeq concentrations, and for PCBs as a sum of individual congeners (higher risk) (USEPA, 2011b).

Risk assessment results for residential exposure to PAHs and PCBs are shown in Table 4.6. Regarding cancer risks, PAH was the group of contaminants showing the highest reasonable maximum exposures in both cities, given by the UCL (95%). The most contaminated site in Lisbon represents a risk of 5.5E-05 and in Viseu the maximum value rises to 2.1E-06. Mutagenic risks of PAHs are higher than cancer risks (Table 4.6), being the reasonable maximum exposure of 3.3E-05 for Lisbon and 3.2E-06 for Viseu. These values are higher than the target excess individual lifetime risk, which is one-in-one-million (10^{-6}) (USEPA, 2011b). For PCBs, cancer risks were always below the target level. For all contaminants and in both cities, the highest risks were found to be from the ingestion exposure route, followed by dermal (always within one order of magnitude) and, at a very low level, the inhalation exposure. Comparing with other studies, Lisbon showed slightly higher total risks for BAPeq than the ones found in Tarragona or in Beijing (for normal conditions) (Nadal et al., 2011; Peng et al., 2011a). The total risk for PCBs is similar for the two cities and also similar to that found in Tarragona (Nadal et al., 2011).

For adults, the cancer risks were also calculated for occupational land use (outside workers, e.g. gardeners). Only PAH levels in Lisbon may represent some concern (Table 6 of Annex III), being the UCL (95%) of 2.4E-06 and the maximum value observed 1.5E-05. In recreational areas, cancer and mutagenic risks were calculated only for PAHs (Table 7 of Annex III) since for other compounds the potential risk should be negligible due to low concentrations. The cancer risk for the more contaminated local, which is in fact a recreational area, was 4.1E-06 and the UCL (95%) was 4.3E-07. However, mutagenic risks are higher, with the most contaminated site representing a risk of 1.2E-05, whereas the UCL (95%) is 1.3E-06. For Viseu, risks were always below 10^{-6}.

These results indicate that the total lifetime carcinogenic or mutagenic risk of PAHs in Lisbon may represent some concern, especially for residential and occupational land use. Indeed, PAH contamination in Lisbon requires more attention and further work should be done, such as the use of a larger dataset (which may be important due to the high variability of results) and other approaches to characterize risks (e.g. probabilistic approach in order to evaluate variability and uncertainty). In addition, site-specific data should be obtained in terms of exposure assessment. The risks could also be more representative if the oral and inhalation bioaccessibility of samples that exceed the target value was determined. This would provide a more substantiated overview of PAH risks in Lisbon urban soils.

Table 4.6 - Concentrations of HOCs (C_{soil}) and results for ingestion (Ing), dermal (Derm), inhalation (Inh) and total cancer risks for residential land use, in both cities. Mutagenic risks of PAHs are also shown.

	Lisbon					Viseu				
	C_{soil} (µg kg^{-1})	Ing	Derm	Inh	Total	C_{soil} (µg kg^{-1})	Ing	Derm	Inh	Total
PAHs - Cancer										
Mean	229	2.6E-06	1.1E-6	7.6E-11	3.7E-6	24	2.8E-07	1.1E-07	8.1E-12	3.9E-07
Med	61	6.9E-07	2.9E-7	2.0E-11	9.8E-7	11	1.3E-7	5.3E-8	3.8E-12	1.8E-07
Min	0.18	2.1E-09	8.7E-10	6.1E-14	3.0E-9	0.6	7.2E-09	3.0E-09	2.1E-13	1.0E-08
Max	3416	3.9E-05	1.6E-05	1.1E-09	5.5e-05	127	1.5E-06	6.0E-07	4.2E-11	2.1E-06
UCL (95%)	558	6.4E-06	2.6E-06	1.9E-10	9.0E-06	47	5.4E-07	2.2E-07	1.6E-11	7.6E-07
PCBs - Cancer										
Mean	7.0	2.2E-08	9.7E-09	1.2E-12	3.2E-08	4.6	1.4E-08	6.4E-09	7.7E-13	2.1E-08
Med	4.7	1.5 E-08	6.5E-09	8.1E-13	2.1E-08	2.2	7.0E-09	3.1E-09	3.8E-13	1.0E-08
Min	0.25	7.8E-10	3.5E-10	4.3E-14	1.1E-09	0.19	5.8E-10	2.6E-10	3.1E-14	8.4E-10
Max	35	1.1E-07	4.8E-08	5.9E-12	1.6E-07	15	4.6E-08	2.1E-08	2.5E-12	6.7E-08
UCL (95%)	9.0	2.8E-08	1.2E-08	1.5E-12	4.0E-08	8.5	2.6E-08	1.2E-08	1.4E-12	3.8E-08
PAHs - Mutagenic										
Mean	229	9.4E-06	4.3E-06	1.9E-10	1.4E-05	24	1.2E-06	4.6E-07	2.0E-11	1.6E-06
Med	61	2.5E-06	1.1E-06	5.1E-11	3.6E-06	11	5.5E-07	2.1E-07	9.5E-12	7.7E-07
Min	0.18	7.5E-09	3.5E-09	1.6E-13	1.1E-08	0.6	3.1E-08	1.2E-08	5.3E-13	4.3E-08
Max	3416	1.4E-04	6.4E-05	2.9E-09	2.0E-04	127	6.2E-06	2.4E-06	1.1E-10	8.6E-06
UCL (95%)	558	2.3E-05	1.0E-05	4.7E-10	3.3E-05	47	2.3E-06	8.8E-07	4.0E-11	3.2E-06

Chapter 5

PAHs IN STREET DUSTS: SOURCE IDENTIFICATION AND IMPACTS ON HUMAN AND ENVIRONMENTAL HEALTH

5.1 INTRODUCTION

Dust is a general term used to describe solid particles below 500 μm, resulting mainly from deposition of airborne material (Lorenzi et al., 2011). However, urban street dusts may be formed not only by deposition of airborne particles, but also by other particles with different origin such as soil derived material, construction materials, weathered materials of pavements and from automobiles (tire debris, brake dust, rust and tail pipe exhaust, fuel exhaust particles). Hence, sources can be natural, telluric or biogenic, and anthropogenic.

Contaminants present in street dust can go back to the atmosphere by evaporation or by wind raise of small particles. On the other hand, dusts can be washed off and drained into the pipe system or directly to nearby aquatic bodies (Boonyatumanond et al., 2007; Brown and Peake, 2006). Therefore, dust contamination may affect other media such as air, drinking water, soil, and plants with a significant exposure risk for human populations. Due to their low particle size, they may represent a higher risk to human health since they are easily transferred through the aforementioned pathways (ingestion, inhalation and dermal contact). In coastal cities like Lisbon, street dusts may also be an important diffuse source through runoff into aquatic environment.

As a result of their primary sources, the major input of PAHs into soils is through atmospheric deposition of contaminated particles (Nam et al., 2009; Ravindra et al., 2008). Nevertheless, street dusts may also contribute to the enrichment of these contaminants in urban soils, since they can be enriched with PAHs (Boonyatumanond et al., 2007; Oda et al., 2001). The potential PAH sources in road dusts are, for example, vehicle exhaust, tire debris, pavement (asphalt or bitumen) or oil spills.

Source apportionment of PAHs in soils is frequently attempted with ratios between single compounds or molecular markers. Nevertheless, the use of ratios has been questioned, since characteristic ratios may shift between emissions and recipient matrices due to differences of physico-chemical properties and degradation rates of individual PAHs (Brandli et al., 2008). Therefore, due to weathering processes it is very difficult to address sources of PAHs contamination in soils, being expected that street dusts will conduct to a more robust identification.

Results from Chapter 4 allowed the identification of PAHs in Lisbon soils as a potential environment and human health issue. Based on the results of this first study a new sampling campaign was conducted in Lisbon urban area in order to get insight on PAHs contamination of this area. Street dusts were also collected in an attempt to better understand sources of PAHs in urban areas and to evaluate the potential risks to the environment and human health posed by

these contaminants in another environmental relevant matrix. Therefore, the aim of this chapter was to assess the levels of PAHs in street dusts from the Lisbon urban area, in order to better identify the major inputs of PAH into soils, characterize their spatial distribution and assess their potential risks.

5.2 MATERIALS AND METHODS

5.2.1 Sampling and analysis

A total of 49 sites were sampled for soil and street dusts, considering different land uses (roadsides, airport sites, ornamental gardens, parks and open spaces, schoolyards and playgrounds) in Lisbon urban area. Sampling location maps shown in Figure 5.1 were produced with ArcGis® Software (version 9.3).

Soil samples were collected and processed as described in section 4.2.1. Dust samples were collected with a brush and a pan and sieved to <0.5 mm. The general characterization of soil samples included pH, organic matter (OM) and organic carbon (OC) content, cation exchange capacity (CEC) and particle size distribution as described in section 4.2.1. The pseudo-total content of PTEs (As, Co, Cr, Cu, Hg, Ni, Pb and Zn) in soil and dust samples were determined as previously described in section 4.2.1.

PAH in soils were determined as described in section 4.2.2 . Due to the low particle size of dust samples some problems were observed when extracting samples by Soxhlet as performed for soil samples, since extracts presented a very high quantity of suspended solids. For that reason dust samples were extracted by sonication as described by Caetano et al. (2012), with some minor modifications. Briefly, about 5 g of dust were extracted with 10 mL of a hexane:acetone mixture (2:1, v/v) in an ultrasonic bath for 20 minutes, centrifuged and decanted. The procedure was repeated three times. Cleanup of extracts for both soil and dust samples was performed as described in section 4.2.2. QA/QC procedures included for both methodologies were also the same as described previously and results were within the same range. Moreover, the variability observed when comparing samples extracted using both methods (Soxhlet and sonication) was <20%.

5.2.2 Statistical analysis

Univariate statistical methods, cluster analysis (CA) and the 95% upper confidence level (UCL) of the mean (used for risk assessment analysis) were calculated has described in section 4.2.4, except that missing values were now considered as equal to half of the detection limit. The Wilcoxon Signed Ranks and t-test were used to test differences between dust and soil. Factor analysis (FA), was applied to obtain underlying factors that describes the covariance among variables. The factors extracted allows the interpretation in accordance with their hypothetical origin (natural, anthropogenic or mixed), and thus groups with common characteristics (e.g. the same origin) can be obtained. Due to differences in units of measurement, FA was conducted in a correlation matrix. The "Kaiser-Meyer-Olkin Measure of Sampling Adequacy test" and the "Bartlett test of Sphericity" were performed in order to measure if the distribution of results is adequate to conduct FA. The extraction method was the principal component analysis (PCA) and the rotation method was the Varimax with Kaiser normalization. All statistical tests were performed in SPSS©.

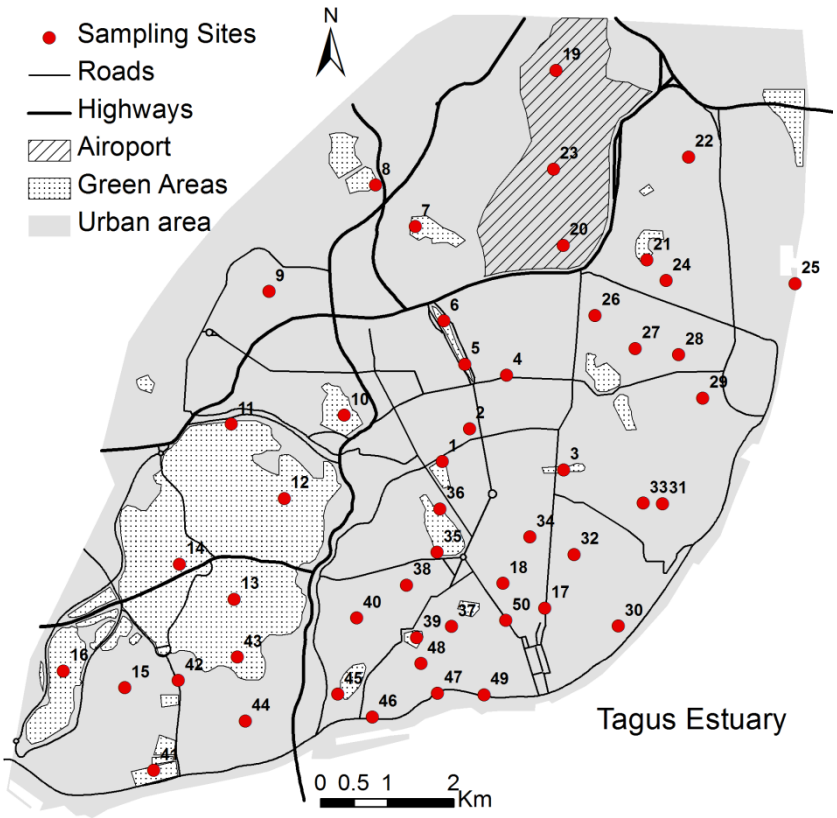

Figure 5.1 - Location of the sampling sites from the second sampling campaign in Lisbon urban area.

5.3 RESULTS AND DISCUSSION

5.3.1 Levels of PAHs in street dusts and soils

Results for the general characterization of soils from this second sampling campaign are presented in Table 1 of Annex IV. The descriptive statistics for the levels of the 8 PTEs found in soils and dusts are also presented in Table 2 and Table 3 of Annex IV, respectively. The mean, median and range of concentrations of the ∑16 PAHs in dust samples is presented in Table 5.1 and Figure 1a of Annex IV, whereas Figure 5.2a shows their spatial distribution within Lisbon urban area. For soils (Figure 5.2b and Figure 1b of Annex IV), concentrations were slightly higher than for dusts, with exception of the maximum value: mean of 3,888 μg kg^{-1} and a median of 714 μg kg^{-1}, ranging from 20 to 73,395 μg kg^{-1}. Comparing the soil concentrations with the previous study conducted in Lisbon (section 4.3.2), the mean, median and maximum values were now much higher, mainly due to the hotspots observed in the airport (Figure 5.2b). Moreover, in the present study sampling was focused in city centre where the concentrations were higher.

For dusts, in addition to the airport, higher concentrations were observed close to the river, in an area nearby the docks, a busy road and the railway line (samples 40, 45, 46 and 48; Figure 5.2a). In general, the spatial distribution of PAHs in soils and street dusts are similar (Figure 5.2), with the highest concentrations observed in the airport and in the city centre. Indeed, the centre of the downtown was already identified as an hotspot of PAHs contamination in street dusts from other urban areas (Wang et al., 2011). In spite of the similar spatial distribution, the concentrations in soils may not be directly related with dust samples, being the Spearman correlation observed between PAHs in soils and street dusts of 0.634 (ρ<0.01). However, the highest concentrations found in soil samples, compared with street dusts, may indicate that soils are the ultimate sink of HOCs in urban areas.

A comparison of levels observed in street dusts with other cities is shown in Table 5.1. Yet, this comparison is very difficult due to the different size fractions sampled in the different studies and also due to the sampling procedure (e.g. vacuum cleaner vs. brush and a pan). Even so, mean concentrations of PAHs in Lisbon street dusts are generally lower than observed in other studies, but the maximum value is one of the highest presented. In fact, the two highest hotspots were observed in the Lisbon International Airport (Figure 5.2a and Figure 1a of Annex IV), but excluding these two sites, the maximum values goes down to 3,743 μg kg^{-1}. Most of the studies presented were conducted in Asian countries, were urban areas are likely to be more affected by PAHs contamination, due to the energy consumption profiles and its low efficiency. Results can be quite different even for the same country, and this can be related, in addition to differences in sources,

to sampling and pre-treatment procedures, and to the choice of sampling locations. Some of the studies were focused on the identification and characterization of PAH sources, and as a consequence samples were collected nearby probable sources not giving a picture of the entire urban area. For example, Oda et al (2001) collected the samples in a roadway tunnel (which is reflected by the low range of results presented) whereas in other studies samples were collected nearby roads or industrial areas (Han et al., 2009; Lee and Dong, 2010). Therefore, the high variability of results observed in Lisbon urban area comparing with other studies is probably due to sampling designs since the present study was focused in sampling areas where human exposition is likely to occur, rather than proximity to sources.

Table 5.1 - Median, mean concentration and range of PAHs (µg kg^{-1}) in street dusts around the world.

City	Fraction (mm)	Median	Mean	Min	Max	Reference
Lisbon	<0.5	671	3,630	71	84,321	This study
Newcastle, UK	<2	-	-	600	46,000	Lorenzi et al., 2011
Dunedin, New Zealand	<2	-	4,400±1,450	-	-	Brown and Peake, 2006
Niterói, Brazil	<1	-	694[b]	434	1247	Netto et al., 2006
Cairo, Egypt	0.045-0.106	-	-	45	2,600	Hassanien and Abdel-Latif, 2008
Egypt (8 cities)	<0.2	-	130[b]	27	379	Mostafa et al., 2009
Tehran, Iran	<0.063	230	330	130	1,410	Saeedi et al., 2012
Amman, Jordan	NS[a]	-	-	13,240	49,960	Jiries, 2003
Kurashiki, Japan	<2	-	23,000±2,000[c]	20,000[c]	25,000[c]	Oda et al., 2001
Chang-Zhu-Tan, China	<0.074	-	8,760	3,515	24,488	Long et al., 2013
Guangzhou, China	<0.1	-	4,800	840	12,300	Wang et al., 2011
Dalian, China	-	-	7,460[d]	1,890[d]	17,070[d]	Wang et al., 2009
Shanghai, China	<0.3	-	20,648[f] 14,098[g]	9,176[f] 6,875[g]	32,573[f] 27,766[g]	Liu et al., 2007
Shanghai, China	<0.074	8,520	8,480	3,180	17,090	Ren et al., 2006
Beijing, China	<0.5	-	-	230	1,210	Xiang et al., 2010
Xincheng, China	<0.9	-	-	1,629	8,986	Zhao et al., 2009
Anshan, China	<0.16	-	144,250[e]	48,730[e]	638,260[e]	Han et al., 2009
Yangtze River Delta, China	<0.15	10,300	15,600	2,240	58,200	Shi et al., 2013
Taichung, Taiwan	<0.3	-	-	16,100	65,800	Fang et al., 2004
Ulsan, South Korea	<2	-	-	19,690	154,640	Lee and Dong, 2010
Kuala Lumpur, Malasia	<0.6	-	224±108	-	-	Omar et al., 2002

[a]Not specified; [b]Geometric mean; [c]∑21PAHs; [d]∑25PAHs; [e]∑11PAHs; [f]winter; [g]summer

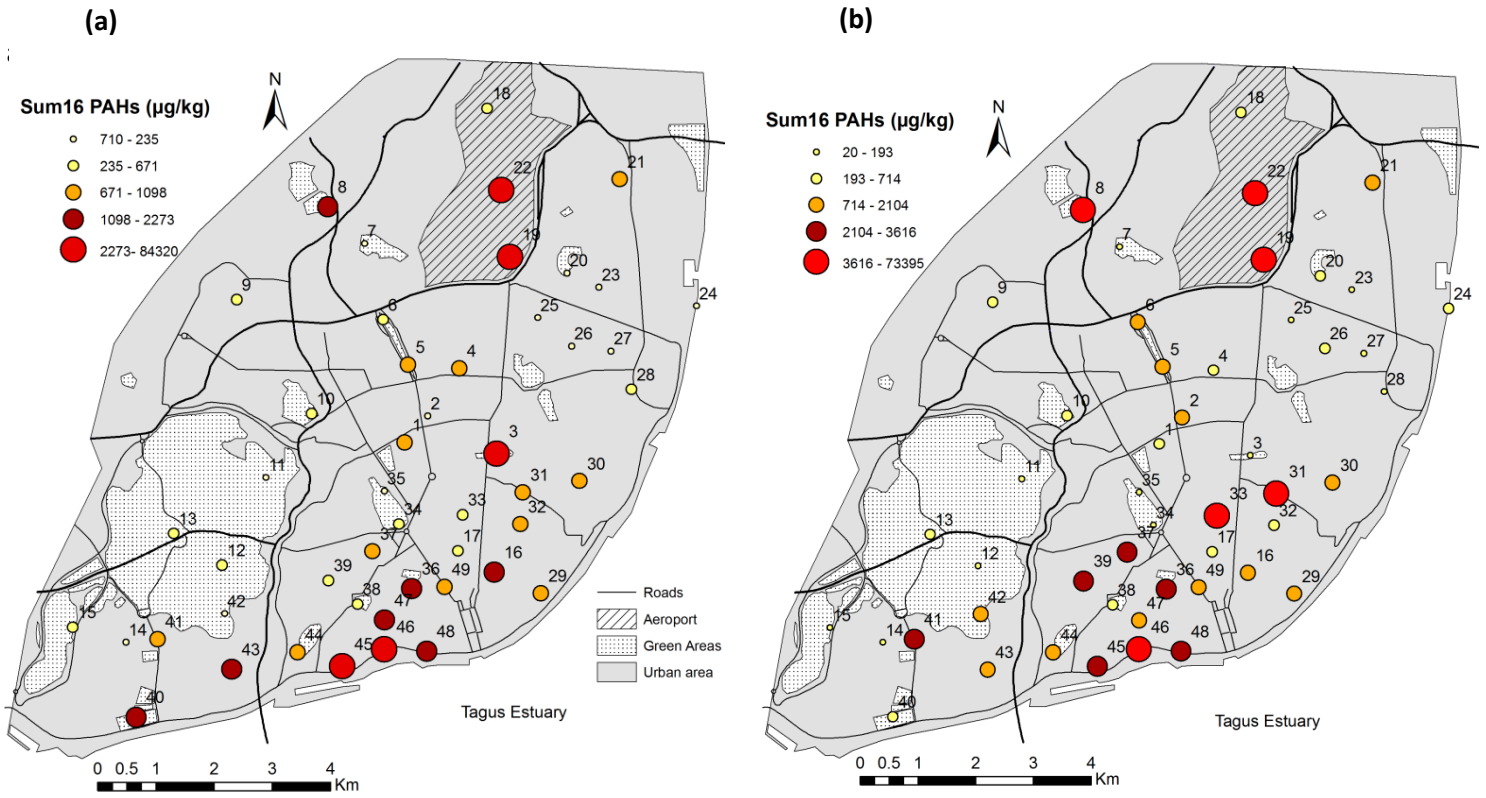

Figure 5.2 - Spatial distribution of PAHs in street dusts (a) and soils (b) from Lisbon urban area. Levels correspond to the minimum, quartiles (25, 50 and 75), upper outlier limit and maximum value.

5.3.2 Source apportionment: PAHs profiles and isomer ratios

PAHs concentrations can vary widely even for the same source type (Ravindra et al., 2008) and therefore profiles are normally used for source identification. The low molecular weight (LMW) PAHs (<4 rings) have primarily a petrogenic origin (unburned fuel emitted from vehicle exhausts, leak of engine oil and fuel, tire debris, weathered materials of road surfaces such as asphalt), whereas for high molecular weight (HMW) PAHs (≥4 rings) the major origin is pyrogenic (incomplete combustion of fuel). Yet, in addition to a petrogenic origin, LMW are also produced by combustion at low to moderate temperatures. For example, Miguel et al. (1998) in a study on the emissions in a tunnel from San Francisco Bay, found that diesel trucks were the major source of LMW PAHs, whereas light-duty gasoline vehicles contribute more with PAHs with ≥4-rings. Yet, 4-ring PAHs have also been related with diesel emissions (Boonyatumanond et al., 2007; Lee and Dong, 2010).

The predominance of HMW PAHs, as observed in present study for both soils and dusts (Figure 5.3 and Figure 2 of Annex IV), is typical from urban areas and it is mostly related to the predominance of pyrogenic over petrogenic sources as explained in section 4.3.2. Indeed, soil profiles were very close to the ones observed in the first survey (Figure 4.3). In addition, these profiles have been also related to the higher persistence of HMW compounds (higher resistance to biodegradation, lower vapour pressure, water solubility, fugacity ratio, volatility and atmospheric mobility), and, since they are normally associated with particulate matter emission, there is a tendency to accumulate in soils that are close to emission sources (Boonyatumanond et al., 2007; Chung et al., 2007; Liu et al., 2010b).

Few studies compared soil and street profiles and for example Wang et al. (2009) did not observe significant differences between concentrations or profiles from the two matrices. Yet, in the present study it was observed that soils were enriched in compounds with 5 and 6 rings (48±9%) comparing with street dusts (41±7%), being the difference statistically significant (p<0.05) (Figure 2 of Annex IV). These results support the hypothesis of the long term accumulation of PAHs in urban soils since these compounds are hardly to be evaporated and degraded. Indeed, Bozlaker et al. (2008), after applying fugacity calculations in air and soil, concluded that the soil acts as a secondary source to the atmosphere for LMW PAHs in summer and as a sink for the higher molecular weight ones in summer and winter, in Izmir urban area (Turkey). On the other hand, LMW PAHs showed a higher percentage in street dusts (15±5%) over soil samples (10±7%), being the difference also statistically significant (p<0.05, Figure 2 of Annex

IV), and suggesting a more recent contamination of this matrix since these compounds are normally present in gaseous form and they are easily evaporated and degraded. Yet, this difference can also be related to specific sources of PAHs in dust samples: wood and coal combustion (domestic heating, forest fires) and diesel engines from domestic heating and from the traffic (especially heavy-duty diesel trucks) (Boonyatumanond et al., 2007; Ravindra et al., 2008; Wilcke, 2007). Therefore, LMW PAHs are related with ubiquitous atmospherically dispersed emissions and, due to their properties, they are subjected to longer distance transport. For the 4-ring compounds there was no significant difference between the two matrices, even that a slightly higher value was observed for dusts (44 % comparing with 41% observed for soils) (Figure 2 of Annex IV).

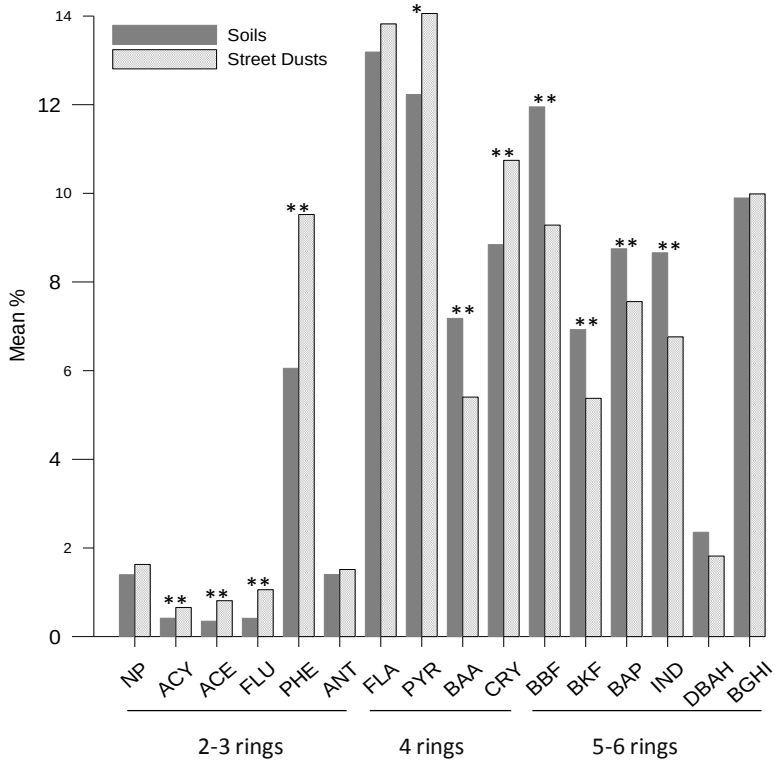

Figure 5.3 - Mean percentages of individual compounds in soils and dusts. Statistical significant differences between the two matrices are signed: * for $p<0.05$ and ** for $p<0.01$.

The high variability of percentages of individual compounds observed for both dusts and soils (Figure 5.4) reflects existence of several profiles. In the particular case of street dusts, multiple source material (atmospheric aerosol, unburned oil, asphalt, tire particles) contribute to the origin of PAHs resulting in multiple PAHs signatures and turning very difficult the use of individual

compounds as tracer of a specific source (Boonyatumanond et al., 2007). Besides, the profiles given in literature for the same source can be different and this difference may be greater due to sampling locations rather than due to differences in size fractions and sampling times (Murakami et al., 2005; Pengchai et al., 2004). Nevertheless, profiles observed for Lisbon street dusts were not very different than the ones from other cities such as Bangkok (Boonyatumanond et al., 2007), Washington (Brown and Peake, 2006), Guangzhou (Wang et al., 2011), Kuala Lumpur (Liu et al., 2007), or Shangai (Omar et al., 2002).

Regarding individual compounds, both dust and soil profiles were dominated by FLA and PYR, which result mainly from fossil fuel (diesel), coal and biomass combustion (Figure 5.3 and Figure 5.4), as explained in section 4.3.2. Yet, all compounds with 5 and 6-ring were enriched in soils, being the differences statistically significant for all except DBAH and BGHI. On the other hand, 2 and 3 ring PAHs (except NP and ANT) showed statistically significant higher values in street dusts.

Several authors (Boonyatumanond et al., 2007; Pengchai et al., 2004) concluded that in addition to vehicle exhaust, tire debris were the major contributors of PAHs to street dusts. Some studies concluded that PYR, FLA, CRY, and BGHI can have origin in tire debris (Boonyatumanond et al., 2007; Glaser et al., 2005) and in fact these compounds were present at higher percentages in Lisbon street dusts. Particularly, the extremely high percentage of PYR in sample 40 (Figure 5.4), which was a playground, could be related to the ground material (granulate rubber flooring made from recycled rubber granules from used tires).

Also the pavement can be a source of PAHs (Pengchai et al., 2004), with different profiles observed in different types of pavements (concrete vs. asphalt) or traffic speed (higher speed increase HMW PAHs) (Lee and Dong, 2010). Yet, these authors state that the higher percentage of PAHs observed in concrete highways is probably due to a higher tire abrasion and concluded that the profile observed (5-ring>4-ring>6-ring>3-ring) should be related to tire debris. Asphalt dusts showed the same trend, but with a higher percentage of 3-ring PAHs (6%), and similar values between the other groups (29-35%). Indeed, the higher percentage of LMW PAHs in street dusts, and especially the very high percentage of PHE may be related with asphalt pavement (Boonyatumanond et al., 2007; Brown and Peake, 2006; Lee and Dong, 2010). In addition to PHE, NP, PYR, CRY, BGHI, BBF, BKF and FLA were also identified as major components of asphalt. Brown and Peake (2006) also presented the profile of used crankcase oil, which is dominated by PHE followed by ANT, NP and PYR.

Concluding, the profiles observed for dusts samples in the present study indicate that tire debris and asphalt may be major contributors to the presence of PAHs in Lisbon street dusts.

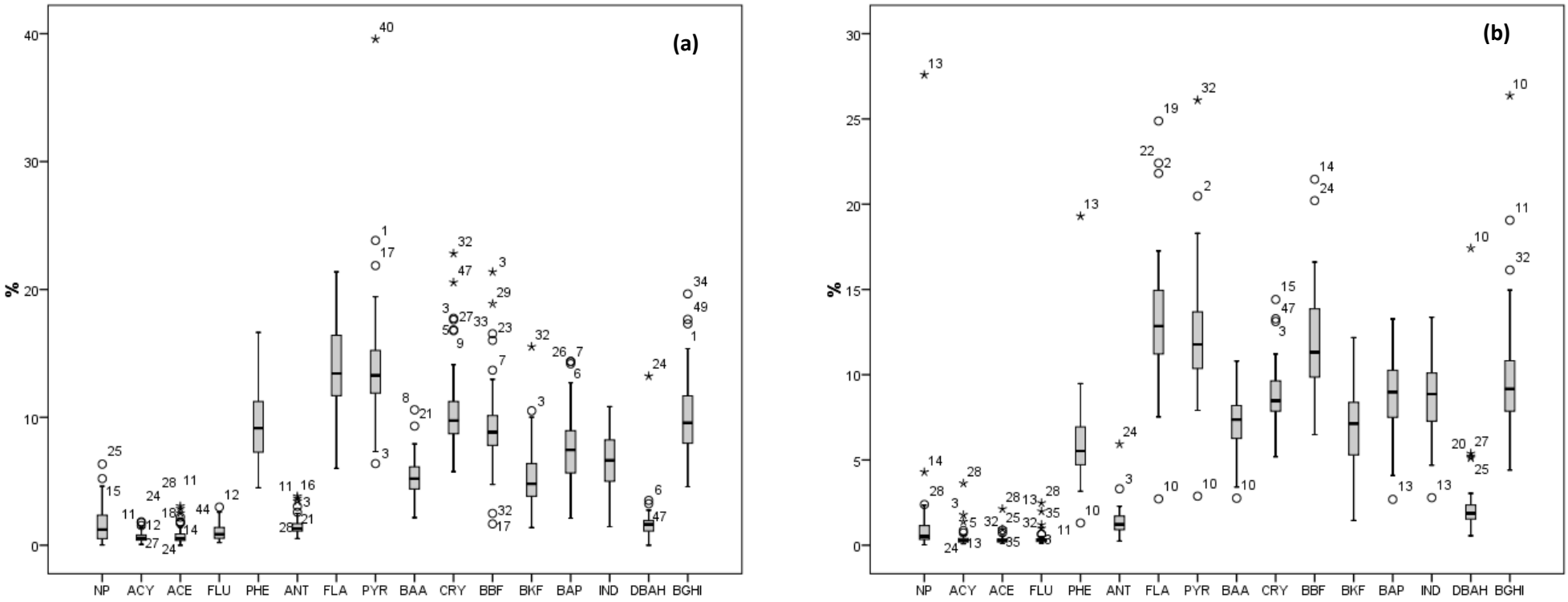

Figure 5.4 - Boxplots of percentages of individual compounds in street dusts (a) and soil samples (b). Boxes define the interquartile range and the line is the median. Outliers are defined as values between 1.5 and 3 box lengths (○) and extreme values as more than 3 box lengths (⋆).

The difference between soil and dust profiles can also be observed in the dendrograms presented in Figure 5.5. Whereas for soils predominant compounds are clustered together (HMW plus PHE), for dusts two groups can be distinguished: one formed by compounds related to combustion (BAP, IND) and other that in addition to combustion have other sources such as asphalt and tire debris (e.g. CRY, PYR, BGHI and PHE). The differences observed between the two dendrograms may also reflect the more recent contamination of dusts whereas for soils the two clusters observed are more likely to be a result of long term accumulation (weathering processes already occurred).

Due to the presence of mixed sources in urban areas and because PAHs composition profiles can be weathered, the use of isomer ratios of selected pairs of isomers (Table 5.2) is commonly applied to address sources. The advantage of the use of ratios of selected pairs of isomers is based on the assumption that confounding factors such as differences in volatility, water solubility or adsorption are minimized. Among the most commonly used are the ones shown in Table 5.2. Yunker et al. (2002) suggest the use of FLA and PYR [FLA/(FLA+PYR)] since the photodegradion of these isomers occurs at similar rate and they show a large range of thermodynamic stability between isomers. Similarly, Zhang et al. (2005) suggests the use of higher molecular weight species such as IND and BGHI [IND/(IND+BGHI)] since they are more stable and less prone to differences in transport or compounds behaviour. Since these ratios are normally calculated as the ratio between one of the compounds and the sum of the isomers, for practical reasons it is normally presented in the denominator of the equation the molecular ion of those isomers, for example, the ratio [FLA/(FLA+PYR)] is normally presented as FLA/202. In Table 5.2 it is also presented the limits most commonly applied to source apportionment of these ratios according to Yunker et al. (2002) and Brandli et al. (2008). Figure 5.6 represents the results for the two most commonly used isomer ratios in all soil and street samples from Lisbon and the source apportionment according to these authors.

Some differences were observed between the isomer ratios of the two matrices (Table 5.2 and Figure 5.6). In general, mean values were lower in dust samples and a wide range of results was observed. In both cases the mean value of these ratios indicates a pyrogenic origin. When looking to each sample, for soils, only one presented a ratio of FLA/202 that could indicate a petrogenic origin. On the other hand, four dust samples presented isomer ratios that could indicate a petrogenic origin considering this ratio. Considering the ratio IND/276 only one dust sample has a petrogenic origin and none for soil samples.

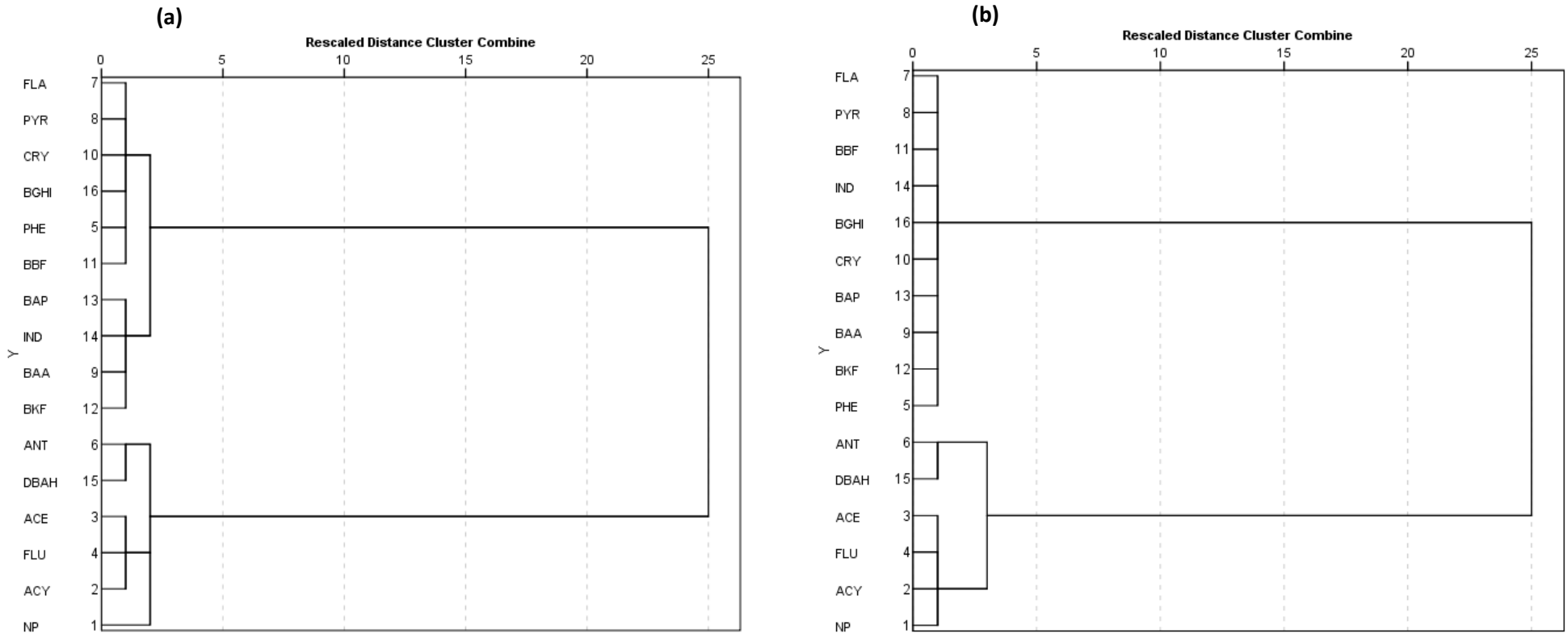

Figure 5.5 - Dendrogram (Ward method, squared Eucleadian distances, log transformed data) of PAH compounds in street dusts (a) and soils (b).

Table 5.2 - Range of isomer ratios observed in the present and in other studies around the world. Mean values, when available, are presented between brackets.

Local	Matrix	PHE/ANT	ANT/(178)	FLA/(202)	BAA/(228)	IND/(276)	Reference
Lisbon, Portugal	Urban soils	1.02-9.21 (4.85±1.6)	0.10-0.50 (0.19±0.07)	0.30-0.58 (0.52±0.04)	0.23-0.55 (0.45±0.07)	0.29-0.54 (0.47±0.06)	This study
	Street dusts	1.74-16 (7.08±2.7)	0.06-0.37 (0.14±0.05)	0.29-0.61 (0.50±0.06)	0.14-0.51 (0.34±0.09)	0.12-0.55 (0.41±0.09)	
Dalian, China	Street dusts	-	>0.1	-	0.35-0.5	-	
Guangzhou, China	Street dusts	-	<0.1	>0.5	0.17-0.40	0.42-0.49	Wang et al., 2011
Shanghai, China	Street dusts	-	0.0-0.26	0.51-0.58	0.38-0.57	-	Liu et al., 2007
Anshan, China	Street dusts	-	-	0.45-0.66	0.39-0.87	0.27-0.32	Han et al., 2009
Egypt (8 cities)	Street dusts	-	0.0-0.40	0.32-0.48	-	-	Mostafa et al., 2009
Taiwan, Taichung	Ambient air	-	-	0.62-0.65	-	0.38-0.50	Fang et al., 2004
Dunedin, New Zealand	Road debris	4.89 (1.09)	-	-	-	-	Brown and Peake, 2006
Lisbon, Portugal	Aerosol particles	-	-	0.48-0.58	0.41-0.52	0.31-0.73	Oliveira et al., 2011
Santiago de Chile	Aerosol particles	-	-	0.43±0.06	0.24±0.07	0.21±0.12	Kavouras et al., 2001
United Kingdom	Ambient air	-	<0.1	>0.5	0.2-0.4	0.4-0.5	Katsoyiannis et al., 2011
-	Oil	-	0.08	0.00	-		Mostafa et al., 2009
-	Used crankcase oil	3.01±1.42	0.22	0.24	-		Boonyatumanond et al., 2007; Mostafa et al., 2009
-	Asphalt	6.26±1.24	0.09	0.43	-	-	Boonyatumanond et al., 2007; Mostafa et al., 2009
-	Tire	8.6±5.6	0.24	0.30	-	-	Boonyatumanond et al., 2007; Mostafa et al., 2009
-	Gasoline soot	2.6±0.67	-	-	-	-	Boonyatumanond et al., 2007
-	Diesel soot	9.34±6.58	-	-	-	-	Boonyatumanond et al., 2007
-	Auto exhaust	-	0.27	0.57	-	-	Mostafa et al., 2009
-	-	>15 petrogenic <10 pyrogenic	<0.1 petrogenic >0.1 pyrogenic	<0.4 petrogenic 0-4-0.5 liquid fossil fuel >0.5 coal and biomass	<0.2 petrogenic >0.2 pyrogenic	<0.2 petrogenic 0-2-0.5 liquid fossil fuel >0.5 coal and biomass	Yunker et al., 2002 Brandli et al., 2008

Isomers ratios are also often used to discriminate between combustion sources. In soils, the FLA/202 ratio pointed to biomass and coal combustion in 88% of samples, whereas according to the IND/276 ratio, 26% of samples have origin in coal and biomass combustion (Figure 5.6). This behaviour was already observed previously (section 4.3.2) and one justification could be the weathering processes that occurred in soils. For dusts, in spite of the similar behaviour observed, 73% of samples showed a FLA/202 ratio that points to coal and biomass combustion, whereas for IND/276 only 12% of samples indicate this origin, the lower percentages observed in both cases may reflect the high contribution of traffic.

Therefore, the fact that different ratios pointed to different sources, even in the case of dusts, raises the question about their applicability to discriminate specific sources. One reason for these contradictory results between ratios can be because FLA and PYR are associated with the combustion of fossil fuel (especially diesel), coal and biomass, whereas BGHI and IND are related with vehicle emissions (especially light-duty), and therefore their use to address specific combustion sources may be not suitable when many sources are present such as in the case of urban soils or street dusts (Boonyatumanond et al., 2007; Oliveira et al., 2011; Ravindra et al., 2008; Yunker et al., 2002). In a literature review of PAHs ratios, Yunker et al. (2002) reports that the FLA/202 ratio ranged between 0.2 to 0.58 for diesel sources, which was very close to the range observed for Lisbon samples (Table 5.2). Therefore, and considering the high contribution of BGHI, BBF and IND (Figure 5.3), which are normally associated with light-duty vehicular emissions, oil combustion or industrial sources, it is probable that the main source of PAH in Lisbon samples is the combustion of liquid fossil fuels (Boonyatumanond et al., 2007; Oliveira et al., 2011; Ravindra et al., 2008).

Other example of isomer ratios can be the isomers BAA and CRY. BAA is photodegraded more easily than its isomer CRY during transportation, and indeed it were observed higher percentages of the last in both matrices. However, it was observed a higher mean ratio of BAA/228 in soils than in dusts, meaning that the higher percentage of CRY in dusts may be related to the presence of sources such as tires and asphalt.

Still, the high variability of results turns difficult to address sources, especially because the values presented for specific sources can be very similar, as can be observed in Table 5.2. This leads to problems such as the limits established to discriminate sources, and as a consequence different values have been reported in literature for the interpretation of ratios and thus the interpretation of isomers ratios is not coherent among studies. For example, several interpretations are given to the ratio BAA/228, with different limits for petrogenic *vs.* pyrogenic

and petroleum *vs*. biomass combustion (Brandli et al., 2008; Liu et al., 2010b; Ma et al., 2009). Other studies (e.g. Oliveira et al., 2011; Ravindra et al., 2008) have a different interpretation for the results of IND/276 than the one of Yunker (2002). Another problem is that different studies used different ratios thus becoming very difficult to compare data (Liu et al., 2010a; Morillo et al., 2007; Peng et al., 2011a). As a matter of fact, the high variability of results observed in the present study turns difficult to compare with the other studies presented in Table 5.2. For example, ratios observed in street dusts from other cities in general showed a lower range of results than in Lisbon, which can be related to the sampling design (studies focused in specific sources). The ratios observed in aerosols from a roadway tunnel from Lisbon, which is used only for light-duty vehicles and consequently the major input is traffic, are closer to the observed in soils than in dusts highlighting the fact that isomers ratio classification should be interpreted with caution (Oliveira et al., 2011).

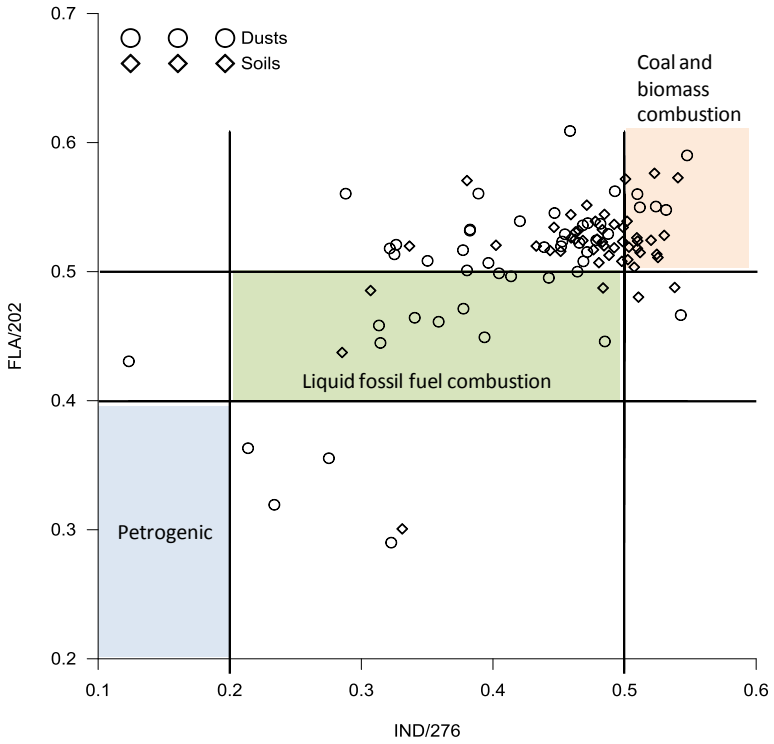

Figure 5.6 - Isomer ratios of dust and soil samples. Colour areas represent an agreement between the two ratios.

5.3.3 Source apportionment: relationship with PTEs

In dust samples the ∑16PAHs showed significant correlations ($\rho<0.01$) with all elements except Al and Ca (Table 4 of Annex IV). For soil samples, significant correlations ($\rho<0.01$) were observed

between ∑16PAHs and As, Ca, Cu, Hg, Pb and Zn (Table 4 of Annex IV) with the highest value observed for Pb (0.766). Soil and dust dendrograms showed some differences (Figure 5.7), with a much more clear distinction of groups in the case of soils. In both cases it was observed a group that included anthropogenic elements (Cu, Pb and Zn) and PAHs and a group that included typical telluric elements (Al, Fe, Co, Cr and Ni) (Cachada et al., 2013). Calcium, which can have origin in coal combustion, cement production or incineration, is clearly grouped with anthropogenic elements in the case of soil samples, but for dust samples it is clustered alone but closer to telluric elements. Arsenic showed the opposite behaviour: clustered together with Hg in dust samples and alone in soil samples but in both cases closer to anthropogenic elements. These differences indicate the complexity of dust samples which can be originated from several sources. Indeed, FA (Table 5.3) gave similar results to CA but it allowed a better understanding of the relationship between contaminants. In the case of dusts it was observed high loadings of anthropogenic elements (Cu, Pb, and Zn) in factor 1 together with PAHs, indicating a major common origin. In fact, Zn has been related to wear and tear of tyres, Cu from brakes linings and electrical wires, whereas Pb is mainly related with leaded fuels (Ajmone-Marsan and Biasioli, 2010; Tijhuis et al., 2002; Zheng et al., 2008). Mercury also shows its highest loading in this factor, but it is only 0.53 and the communality value is also low indicating that it can be related with other factors. As a matter of fact, sources of Hg are mainly related to industrial activity but emissions can also be related with smelting and combustion of fossil fuels (Ajmone-Marsan and Biasioli, 2010; Cachada et al., 2012b). A similar behaviour (low loadings and communalities) was observed for Ca but in this case the highest loading was on factor 2, which is related to a telluric origin. Arsenic has similar loadings in both factors, which can be addressed as both anthropogenic and telluric origin, yet the communality of this element is also very low. In addition, Cr and Ni, two typical telluric elements from Lisbon urban area, show significant loadings on factor 1 (>0.5). However, the presence of these contaminants may also be related to traffic (Ajmone-Marsan and Biasioli, 2010).

These ambiguities found in FA reflect the different origins of the dusts such as soil derived material, atmospheric particles, tire and pavement debris. Looking to FA of soil samples (Table 5.3) the behaviour of contaminants is much clear, being easy to identify the telluric and the anthropogenic factors and the contaminants loaded in each one. For soils, typical anthropogenic elements (Cu, Hg, Pb and Zn), Ca and the ∑PAHs show very high loadings in factor 2, whereas telluric elements (Al, Fe, Co, Cr and Ni) show high loadings on factor 1. This factor also shows a high negative loading of As, however this element it is also related with factor 2.

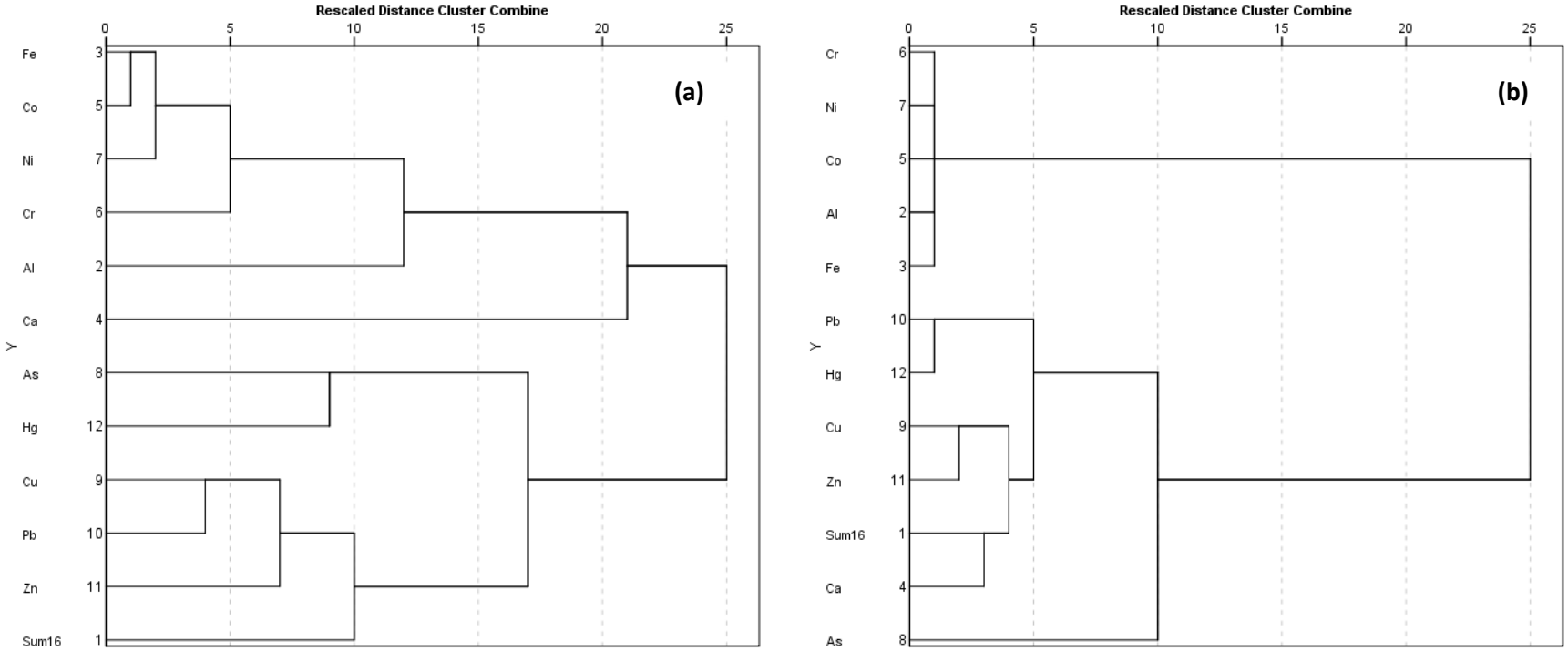

Figure 5.7 - Dendrogram (Ward method, squared Eucleadian distances, log transformed) for street dusts (a) and soil samples (b).

Table 5.3 - Factor loadings of PAHs and PTEs, after Principal Component extraction and Varimax rotation. Coefficients higher than 0.5 are marked in bold. Communalities, eigenvalues and the cumulative variance explained by the factors, are also shown.

Variable	Dust			Soil		
	Factor 1	Factor 2	Communality	Factor 1	Factor 2	Communality
∑16PAHs	**.845**	.020	.715	.006	**.845**	.713
Al	.091	**.817**	.676	**.916**	.219	.888
Fe	.407	**.844**	.878	**.946**	.272	.969
Co	.349	**.839**	.825	**.931**	.251	.931
Cr	*.593*	**.614**	.728	**.953**	.263	.978
Ni	*.525*	**.731**	.810	**.943**	.290	.973
Cu	**.819**	.375	.812	.379	**.800**	.785
Pb	**.808**	.375	.817	.163	**.932**	.895
Zn	**.749**	.285	.642	.425	**.762**	.761
Hg	**.533**	.368	.420	.050	**.803**	.647
Ca	.134	**.510**	.278	.266	**.757**	.644
As	*.483*	**.487**	.471	**-.705**	.454	.702
Eigenvalue	6.9	1.2	-	6.9	3.0	-
% Cumul. Expl. Var.	57	67	-	57	82	-

5.3.4 Potential risk of PAHs in street dusts to the environment and human health

Since the potential risks of soils samples were addressed in Chapter 4 and will be further evaluated in Chapter 6, this section will be focused on potential impact of dusts to the environment and human health. Even that soil samples are the ultimate sink of PAHs, resulting in a detrimental quality; dust samples could have a stronger impact due to their environmental significance. Dusts contamination may affect other media such as soils, air, drinking water, plants, animals and human populations. Street dusts may be an important diffuse source through runoff into aquatic environment resulting in an enrichment of sediment's levels, being this phenomenon especially important in riverine or coastal areas such as Lisbon. Regarding human health, in addition to a more direct contact with human population than soils, they also tend to be more easily transferred through ingestion, inhalation or dermal contact due to their low particle size.

5.3.4.1 Potential risks to the environment health (ERA)

Once there are no guidelines for levels of contaminants in dusts, the assessment of potential risks to the environment is difficult and more specific data should be obtained (e.g. through ecotoxicological tests). However, considering that one relevant pathway of dusts to wildlife is the ingestion, the levels of Lisbon street dusts were compared with recommend levels for the

ingestion of soil and food for the protection of livestock and wildlife from the Canadian guidelines (Table 2.8). Therefore, the hazard quotients (HQs) and hazard index (HI) based on these guidelines were calculated (Eq. 2.1) and they can be found in Table 5.4. The results show that only the two samples from the airport represent a potential hazard considering this route of exposure (HQ and HI higher than 1).

Another problem concerning the contamination of street dusts by PAHs could be their transport into the aquatic system. Both the Spanish and the Canadian guidelines (Table 2.7 and Table 2.8) present soil quality criteria for the protection of aquatic organisms. However, levels of PAHs in street dusts were compared only with the Canadian since in order to compare with the Spanish guidelines a correction for the OC/OM content should be made but, these properties were not determined in dust samples. The results of the aforementioned comparison (HQ and HI) with Canadian quality guidelines for protection can found in Table 5.4. The highest HQ observed was for PHE regarding the freshwater protection, being the median value above one. Indeed, 60% of samples showed a HQ>1 for PHE, 14% for NP and 73% of sampling sites showed a HI>1 when comparing with these guideline values. In fact, the levels of PAHs seem to be a potential problem if dusts reach the aquatic environment.

Table 5.4 - Minimum, median and maximum value for the HQ of Lisbon urban dusts according to different Canadian soil screening guidelines.

	Canada – Ingestion			**Canada – Freshwater**		
	min	med	max	min	med	max
NP	0.00	0.00	0.00	0.07	0.58	3.58
ACY	-	-	-	0.00	0.00	0.00
ACE	0.00	0.00	0.01	0.00	0.01	0.80
FLU	0.00	0.00	0.02	0.00	0.02	1.07
PHE	0.00	0.00	0.14	0.14	1.37	128
ANT	0.00	0.00	0.01	-	-	-
FLA	0.00	0.00	1.17	-	-	-
PYR	0.00	0.01	1.91	-	-	-
BAA	0.00	0.00	0.76	-	-	-
BBF	0.00	001	1.41	-	-	-
BKF	0.00	0.00	0.01	-	-	-
BAP	0.00	0.00	0.00	0.00	0.00	0.00
IND	0.01	0.07	8.35	-	-	-
DBAH	-	-	-	-	-	-
∑5PAHs	-	-	-	-	-	-
HI	0.01	0.12	14	0.29	1.88	132

Assuming that the nearby estuary will be a receptor of street dusts, levels can be compared with sediment quality guidelines in order to assess their impact on this compartment. However it should be noted that once in the sediment compartment these levels will be diluted. Comparing the levels of PAHs in Lisbon street dusts with Canadian freshwater or marine Interim sediment quality guidelines for the protection of aquatic life (Table 5 of Annex IV) the exceeded values can reach 67% for PYR, 59% for PHE, 57% for BaP and DBAH, 47% for BAA, 41 for FLA, 29 for ACY and less than 20% for ACE, ANT, FLU and NP. If the probable effect level is considered (Table 5 of Annex IV), which defines the level above which adverse effects are expected to occur frequently, only less than 5% of samples exceed the values both for freshwater and marine sediments. Therefore, and especially considering the interim guidelines, the impact of street dust on the aquatic environment should be further assessed.

5.3.4.2 Potential risks to the human health (HHRA)

As for the environmental health, there are no specific guidelines for dusts. Therefore, levels were compared with soil quality guidelines. The rationale for this comparison is that street dusts, due to their low particle size, tend to be more easily transferred through ingestion, inhalation or dermal contact (which are the routes normally considered in the derivation of these guidelines). Taking into consideration the concentrations recommended by Denmark for BAP, DBAH and ∑5PAHs (Table 2.9), they were exceeded in 20%, 4% and 8% of sites, respectively, indicating that in these areas exposure should be reduced. In addition, two samples were above the cut of value for BAP and ∑5PAHs. Considering the Spanish guidelines (Table 2.11), 5 samples were above the recommended value for BAP, and for the other compounds less than two samples exceed the recommended value for residential areas.

Comparing with the Italian guidelines for residential and public park areas (Table 2.11), 30% of samples exceed the recommended value for BGHI, 20% for BAP, 18% for DBAH but for the other compounds the values were exceeded in less than 6% of samples. Considering the USEPA screening values for residential areas, the most problematic compound is the BAP with 76% of samples with levels above the recommended, followed by DBAH, BBF and IND (with 29, 16 and 14% respectively) and less than 10% for the other compounds. Finally PAH toxic potency of soil samples, expressed as BAP-equivalents (BAPeq) was calculated (Table 6 of Annex IV) in order to enable a comparison with the Canadian guidelines. The ∑BAPeq (mean value) in Lisbon was 409 µg BAPeq kg^{-1} ranging from 9.35 to 8,994 µg BAPeq kg^{-1} (Table 6 of Annex IV), and only 3 dust samples were above the recommended value of 600 µg BAPeq kg^{-1}.

The non-cancer hazard quotient, calculated according to USEPA methodology, is below the target value of 1 for all contaminants which have an estimated RfD_O (Table 2.3). Similarly, using the Dutch methodology the HQs were always below 1.

The potential risks (cancer and mutagenic) of the amount of PAHs present in dusts for the three routes of exposure considered (ingestion, inhalation and dermal uptake) are shown in Table 5.5. The highest reasonable maximum exposure for a resident exposure, given by the 95% percentile of the upper confidence level of the mean (UCL 95%) for both cancer and mutagenic risks are higher than 10^{-5} (1 case in 100,000 inhabitants). However, the default USEPA values can be seen as the worst case scenario and if a lower ingestion rate is considered (50 mg for adult and 100 mg for children) as suggested by the Dutch model (Table 2.2), the UCL (95%) value drops to 1.4E-05. Yet, this value is extremely influenced by the airport samples, and if these samples are not considered the UCL value drop off to 2.17E-06 using the USEPA default values and to 1.4E-06 using the Dutch values. Even so, these values are higher than the target excess individual lifetime risk, which is one in one million (10^{-6}).

Regarding an occupational exposure the UCL (95%) cancer risk is higher than 10^{-6}, and at the airport this values rises to values higher than 10^{-5} in both cases using the USEPA default values (Table 5.5). If the soil ingestion rate is changed to 50 mg according to the Dutch recommendation, the UCL (95%) cancer risk is still higher than 10^{-6} (4.2E-06). For recreational land use, the UCL is lower than 10^{-6}, but seven samples showed mutagenic risks higher than the target value. However, when considering only the samples collected in playgrounds the risks are negligible (Table 5.5).

The results obtained for dust samples are in line with the ones obtained for soil samples (Table 6 of Annex IV), yet the importance of dusts regarding the potential of ingestion, inhalation or dermal exposition is much higher than for soil samples. However, from these three routes of exposure, it is expected that inhalation and dermal contact would be the most important in an urban environment. Yet, as it was observed in section 4.3.5.2, these two routes are the ones representing lower risks. In the particular case of dusts, for the inhalation route, risks are always below the target, whereas for the dermal contact the UCL (95%) is slightly higher than the target considering a residential and occupational exposure. These risks are merely indicative but they can be considered protective since they are based on the worst case scenarios. As referred in the previous chapter, in order to assess the real risk of street dust to the Lisbon population these models should be improved in order to be more realistic and to reduce the uncertainties. Indeed,

site-specific exposure assessment data should be considered as well as an evaluation of the bioaccessible fraction of these contaminants in street dusts.

Table 5.5 - Statistical data of total cancer and mutagenic risks of dust samples, considering different land uses.

	Residential		**Worker**	**Recreational**		**Playgrounds**	
	Cancer	**Mutagenic**	**Cancer**	**Cancer**	**Mutagenic**	**Cancer**	**Mutagenic**
UCL	2.2E-05	8.1E-05	5.8E-06	1.6E-07	6.8E-07	1.6E-07	6.5E-07
med	1.1E-06	4.5E-06	2.8E-07	7.3E-08	3.1E-07	7.3E-08	3.1E-07
mean	6.6E-06	2.8E-05	1.7E-06	1.3E-07	5.3E-07	1.0E-07	4.4E-07
90%	5.3E-06	2.2E-05	1.4E-06	2.8E-07	1.2E-06	2.0E-07	8.5E-07
min	1.5E-07	6.3E-07	4.0E-08	1.1E-08	4.7E-08	1.6E-08	6.6E-08
máx	1.4E-04	6.1E-04	3.8E-05	7.6E-07	3.2E-06	4.1E-07	1.7E-06

Chapter 6

SPATIAL VARIABILITY AND SOURCES OF POLYCYCLIC AROMATIC HYDROCARBONS IN LISBON URBAN SOILS

6.1 INTRODUCTION

Typically, the first step for assessment of soil quality is based on the total content of contaminants in soil and its comparison with threshold values in order to calculate their potential hazard. At this stage of the risk assessment (RA) plan multivariate and geostatistical tools can be very useful for site characterization, for example to evaluate the extent of contamination or to identify sources of contamination. Indeed, source apportioning is an essential step to control and/or reduce the inputs of contaminants to soils. Taking as an advantage the large database obtained for Lisbon urban soils, it was possible to apply geostatistical methods, allowing a better identification of areas of concern (risk maps). These methods consist in a type of applied statistics that focuses on the spatial context and the spatial relationships among data. An interesting aspect of geostatistics is the use of spatial variables that describe phenomena with a geographical distribution. Consequently, spatial analysis tools can be very useful for example to relate risks to an area potentially affected, which may be useful in risk management. In addition, these approaches can be useful to integrate spatial information on contaminant concentrations and land use.

Soils, and especially urban soils, can be very heterogeneous and consequently its properties and concentrations of pollutants may vary remarkably over short distances, being very difficult to have a homogeneous data set. In addition, due to their patchy nature it is not always possible to sample at the desirable location and consequently it can be difficult to obtain a "real picture" of the area. However, unknown values can be estimated from data taken at specific locations by applying geostatistical procedures. This procedures estimate values of a surface at the nodes of a regular grid from irregularly spaced data points. In this context, one of the most useful spatial analytical techniques is kriging interpolation, which is a "family" of non-stationary estimators based on lest-squares regression algorithms (Goovaerts, 1997). This is a linear-weighted gridding method that has been successfully used to produce illustrative contour plots of contaminant distribution on the basis of scattered observed concentration data, i.e., to transfer scatter points into a continuous data surface (Chung et al., 2007; Liu et al., 2007; Peng et al., 2011b).

Kriging interpolation of spatial data, together with multivariate statistical methods can be useful tools to extract additional information from the data set and thus reducing uncertainties and minimising the costs (Candeias et al., 2011; Reis et al., 2012). Therefore, based on results obtained in previous chapters, where it was concluded that Lisbon urban area can represent a potential risk due to high levels of PAHs presented in some areas, it was decided to conduct a further study in which the referred methodologies were applied, in order to better understand the spatial distribution, major inputs and potential risks of these contaminants.

6.2 MATERIAL AND METHODS

6.2.1 Sampling and soil characterization

Fifty one samples were collected in the first sampling campaign (Chapter 4) and fifty in the second one (Chapter 5). In order to evaluate the comparability of the results of both campaigns, and therefore the possibility of joining the data, four composite samples were collected exactly in the same sites during the second campaign, resulting in a total of 97 sites sampled (Figure 6.1). Other samples were collected in the same parks or gardens but not exactly in the same location and in these cases they were treated as different sites. In addition, duplicate samples and side-by-side samples were collected in order to evaluate the spatial variability.

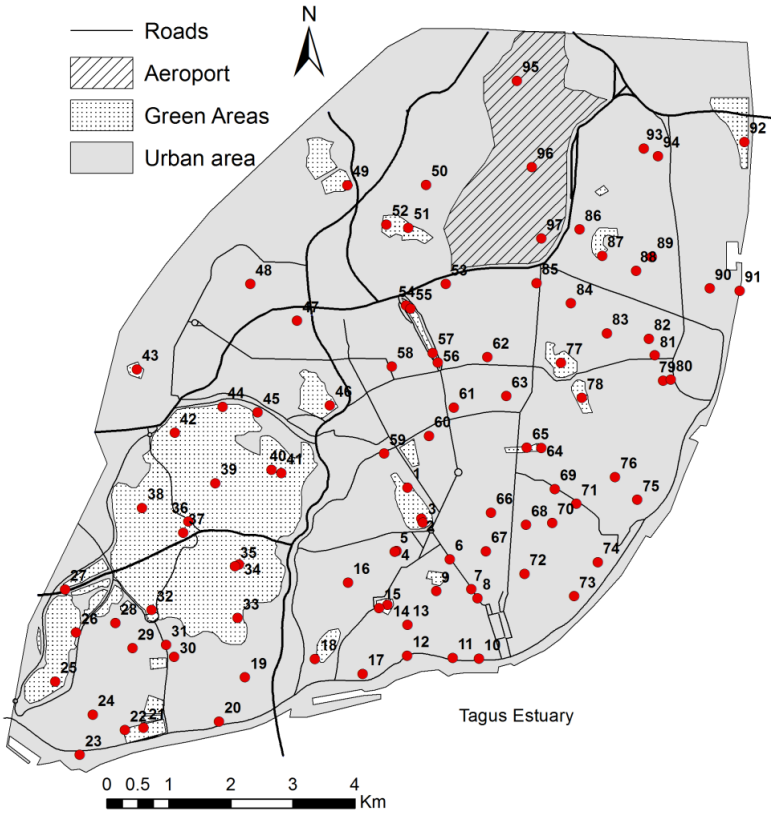

Figure 6.1 - Location of the sampling sites from the two sampling campaigns in Lisbon urban area.

6.2.2 Statistical methods

Univariate and multivariate statistical methods were the same as described previously in Chapters 4 and 5. The variogram ($\gamma(h)$) technique, which is the basic instrument of geostatistics, was used

to measure the spatial variability of variables. A briefly explanation will be given here, based on Goovaerts (1997) and Soares (2000). The variogram relates the semi-variance, half the expected squared difference between paired data values Z(x) and Z(x+h), to the lag distance h, by which locations are separated, as presented in Eq. 6.1. For discrete sampling sites, such as soil samples, the function is estimated as presented in Eq. 6.2, where $Z(x_i)$ is the value of the variable Z at location of x_i, and N(h) is the number of pairs of sample points separated by the lag distance h. In the case of irregular sampling, the distance between the sample pairs is not exactly equal to h, and in this case the lag distance h is represented by a distance band.

Eq. 6.1

$$\gamma(h) = \frac{1}{2}E[Z(x) - Z(x+h]^2$$

Eq. 6.2

$$\gamma(h) = \frac{1}{2N(h)}\sum_{i=1}^{N(h)}[Z(x_i) - Z(x_i + h]^2$$

A variogram plot can be acquired by calculating variogram at different lags and fitted with a theoretical model, such as spherical, exponential, or gaussian. This fitted model will provide information about the spatial structure as well as the input parameters for kriging interpolation.

Ordinary kriging allows to account for local variation by using a theoretical weighted moving average as shown in Eq. 6.3, where $\widehat{Z}(x_0)$ is the value to be estimated at the location of x_0 and $Z(x_i)$ is the known value at the sampling site x_i. It uses n sites for the estimation, which are selected based on the size of the moving window and user definition. The weighting function is not arbitrary, and it is calculated based on the parameters of the variogram model. In order to ensure that the estimate is unbiased, the weights need to sum to one (Eq. 6.4) and the estimation errors (or kriging variances) need to be minimised. In the present study the ordinary kriging interpolation analysis with the spherical model was used to construct the filled contour maps, using ArcGis® Software (version 9.3).

Eq. 6.3

$$\hat{Z}(x_0) = \sum_{i=1}^{n}\lambda_i Z(x_i)$$

Eq. 6.4

$$\sum_{i=1}^{n}\lambda_i = 1$$

6.3 RESULTS AND DISCUSSION

6.3.1 Characterization of soils and comparison between sampling campaigns

Results for the general parameters of the two sampling campaigns can be found in Table 4.1 and in Table 1 of Annex IV, and it's possible to observe that in general the mean and median values were very similar. For PTEs results for the first and second sampling campaign can be found in Table 4.2 and Table 2 of Annex IV, respectively, and again the mean and median values were similar in both campaigns for most of the elements. The comparison of PAHs results was already made in section 5.3.1 and the higher mean, median and maximum values observed in the second campaign was mainly attributed to the airport samples and because it was focused in city centre where the concentrations of PAHs are higher. Results for individual compounds for the different campaigns are shown in Table 1 of Annex V. Considering all the general parameters, the differences observed for the same sites sampled in the different sampling campaigns were within the range of differences observed between duplicate samples (<25%). Therefore, it was assumed that spatial variability was more important than temporal variability and results were gathered in one dataset. The results of general parameters for the gathered data can be found in Table 6.1, whereas for PTEs results can be found in Table 6.2 and final results for individual PAHs compounds are presented in Table1 of Annex V. For PAHs the variability in the levels found in the same sampling sites was high (around 35% for individual compounds), but within the range of duplicate and side-by-side samples. However, the profiles of PAHs were also compared (t-test) and no statistical significant differences were observed between samples.

Table 6.1 - Results of the general parameters determined in Lisbon soils in two sampling campaigns: pH, total carbn (TC), organic carbon (OC), organic matter (OM), cation exchange capacity (CEC) and particle size (sand, silt and clay).

Parameter	Mean	Median	Min.	Max.
pH ($CaCl_2$)	7.0	7.0	5.5	7.6
TC (%)	4.5	4.1	0.69	24
OC (%)	2.7	2.4	0.57	8.6
OM (%)	7.4	6.4	0.91	40
CEC (cmol kg^{-1})	19	18	2.1	52
Sand (%)	60	60	11	96
Silt (%)	26.6	27	2.9	48
Clay (%)	14	13	1.0	44

Table 6.2 - Descriptive statistics of PTEs concentrations in Lisbon urban soils from the two sampling campaigns.

Element	Mean	Median	Min.	Max.
Al (%)	1.23	1.01	0.21	4.27
Fe (%)	2.14	1.77	0.18	7.31
As (mg kg^{-1})	4.74	4.40	<0.1	29.1
Ca (%)	3.97	3.49	0.10	12.7
Co (mg kg^{-1})	11.05	6.50	0.60	48.8
Cr (mg kg^{-1})	41.1	29.1	1.00	201
Cu (mg kg^{-1})	45.7	35.8	3.50	258
Hg (mg kg^{-1})	0.44	0.19	0.01	3.76
Ni (mg kg^{-1})	40.1	21.7	2.00	209
Pb (mg kg^{-1})	89.7	66.4	4.7	428
Zn (mg kg^{-1})	117	100	7.00	540

6.3.2 Levels and sources of PAHs in Lisbon urban soils

Concentrations of the ∑16PAHs obtained in surface soils ranged between 6.3 and 73,395 µg kg^{-1}, with a mean value of 2,645 and a median of 559 µg kg^{-1} (Figure 6.2). The comparison with other urban areas (Table 2 of Annex V) was already discussed in section 4.3.2, being the concentrations found in the present study similar to other European cities and much higher than observed in other Portuguese urban areas.

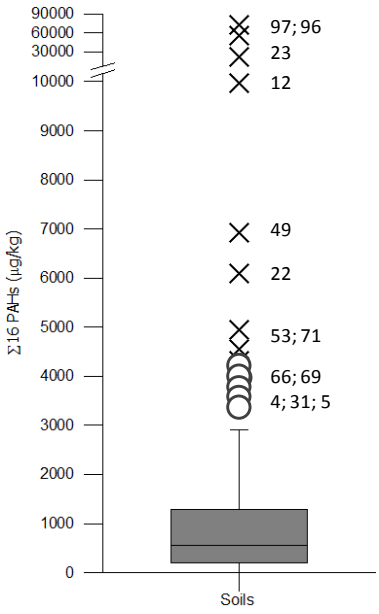

Figure 6.2 - Boxplot showing the variation of the ∑16PAHs in Lisbon soils. Boxes define the interquartile range and the line is the median. Outliers are defined as values between 1.5 and 3 box lengths (○) and extreme values as more than 3 box lengths (x).

The use of the ordinary kriging interpolation method (Figure 6.3) allows a better understanding of the spatial distribution of PAHs in Lisbon urban soils. It is clearly shown that the areas with the highest PAHs concentrations are the airport and the area close to the river (city centre), which is the oldest part of the city with a higher population density (Figure 1 of Annex V). These areas also correspond to the ones where the highest levels of PAHs in street dusts were found (Figure 5.2). The location of some of the specific sources of PAHs in soils from the city centre previously identified in sections 4.3.2 and 5.3.1 (railway, crematoriums, hospital waste incinerator, the docks and shipyard activity) can be found in Figure 6.3. In addition, the former industry present in the city centre cannot be ignored (Cachada et al., 2013). Even so, as concluded in the previous chapters, traffic is a very important diffuse source of PAHs into urban soils, through atmospheric emissions, tire debris and fuel exhaust, as well as pavement debris. Therefore, the high concentrations found in soils from the city centre are essentially a result of long term accumulation in historical parks and gardens due to diffuse pollution. Indeed most of the sites sampled in the city centre were historical gardens and parks such for example samples 9-18, 22-23, and 54-56.

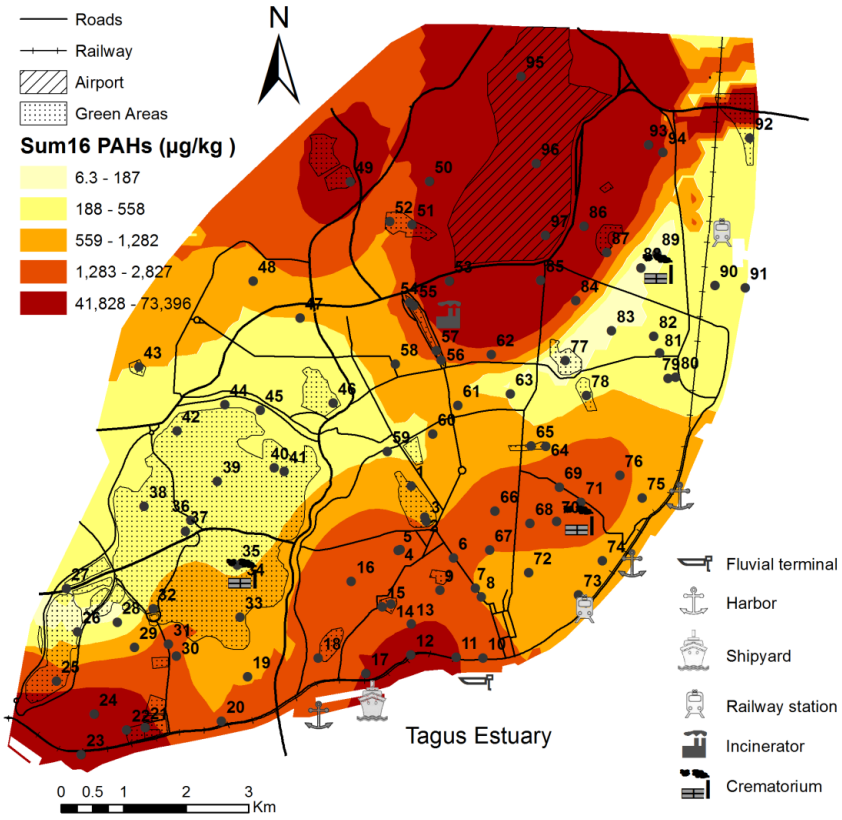

Figure 6.3 – Contour map interpolated by ordinary kriging of the distribution of the ∑16 PAHs; the class limits correspond to the minimum, the quartiles (25, 50 and 75), the upper outlier limit, and the maximum value. Some of most important sources are identified.

As referred previously no statistical significant differences were observed between the PAHs profiles of samples from the two sampling campaigns. Therefore, the profiles presented in Figure 6.4 are coincident to the ones from Figure 4.3 and Figure 5.3. The dominant group are the high molecular weight (HMW) PAHs (≥4 rings), with the 4 ring PAHs showing a median value of 43% and the 5+6-ring 47%, whereas 2+3 ring only showed a median percentage of 9%. Similarly, the most abundant compounds were fluoranthene (FLA), pyrene (PYR), benzo(b)fluoranthene BBF and benzo(ghi)perylene (BGHI) (Figure 6.4), and their sources were already addressed in sections 4.3.2 and 5.3.2. The dendrogram of individual compounds (Figure 2 of Annex V) also remains unaltered when comparing to Figure 5.5, as well as the isomer ratios (Figure 3 and Table 3 of Annex V). With exception of the ratio FLA/278, in one sample, all other ratios indicate that the origin of PAHs in Lisbon soils is pyrogenic. Concerning specific sources, the ratio FLA/202 addresses to coal and biomass combustion in 96% of samples but according to the ratio IND/276 only 28% of samples have this origin.

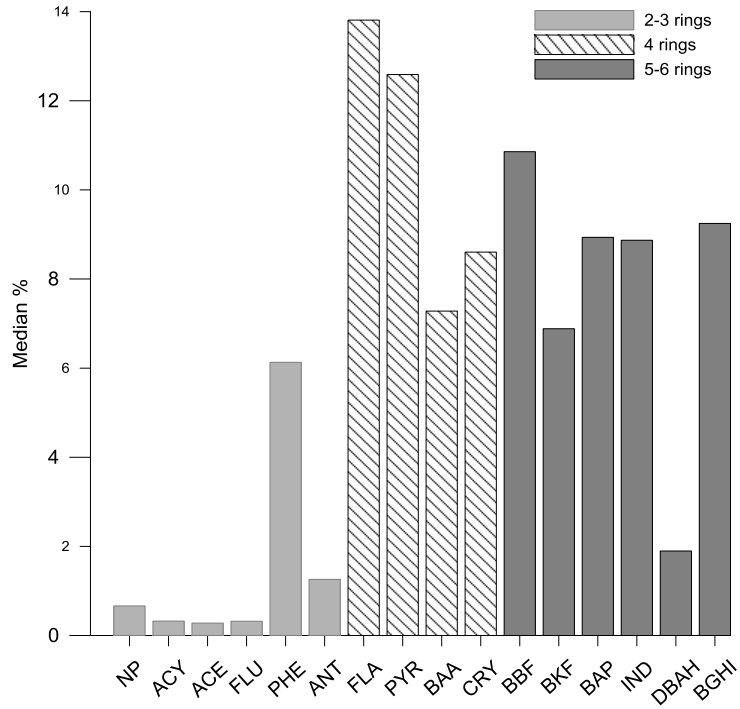

Figure 6.4 - Median percentages of individual compounds in Lisbon urban soils considering the two sampling campaigns.

Significant correlations (ρ<0.01) were observed between the ∑PAHs and As, Ca, Cu, Hg, Pb and Zn (Table 4 of Annex V), with the highest value observed for Pb (0.804). Again, the dendrogram of

PAHs and PTEs (Figure 4 of Annex V) remains similar to the one presented in Figure 5.7, with PAHs clustered together with anthropogenic elements (Cu, Hg, Pb, and Zn). However, in this case PAHs are more close to Hg and Pb than with Cu and Zn. Similarly, the factor analysis (FA) including the ∑16PAHs and PTEs previously identified as having telluric (Al, Fe, Co, Cr and Ni) or anthropogenic (Ca, Cu, Hg, Pb and Zn) origin, resulted in two factors (Table 6.3), as observed in Table 5.3. Arsenic was not considered in the present FA due to its low communality value. Factor 1 can be described as the telluric origin of PTEs, since it has high loadings of Al, Fe, Cr and Ni, and factor 2 is related with anthropogenic origin of both PTEs and PAHs. The presence of Ca in this factor can be related to its origin in coal combustion, cement production and incineration. Copper and Zn, which are normally associated with traffic contamination in urban areas, showed also some influence on factor 1 and this behaviour is reflected on the loading plot shown in Figure 6.5. In this loading plot of both factors it's possible to clearly observe how contaminants are grouped, and to note the presence of two groups for anthropogenic elements: one formed by Hg, Pb and PAHs and other formed by Ca, Cu and Zn. In spite of the common anthropogenic sources of these PTEs and PAHs the separation of these two groups could be related to other specific sources or to the influence of soil properties on the retention of these contaminants as explained in Chapter 4.

Table 6.3 - Factor loadings of PAHs and PTEs, after Principal Component extraction and Varimax rotation. Coefficients higher than 0.5 are marked in bold. Communalities, eigenvalues and the cumulative variance explained by the factors are also shown.

	Factor 1	**Factor 2**	**Communalities**
Al	**.964**	.118	.944
Fe	**.966**	.202	.974
Co	**.950**	.121	.917
Cr	**.933**	.229	.924
Ni	**.949**	.194	.938
Ca	.309	**.650**	.518
Cu	*.441*	**.785**	.810
Hg	.026	**.862**	.744
Pb	.078	**.948**	.904
Zn	*.412*	**.797**	.805
Sum16	.005	**.864**	.747
Eigenvalue	6.5	2.8	-
% Cumulative Expl. Var	59	84	-

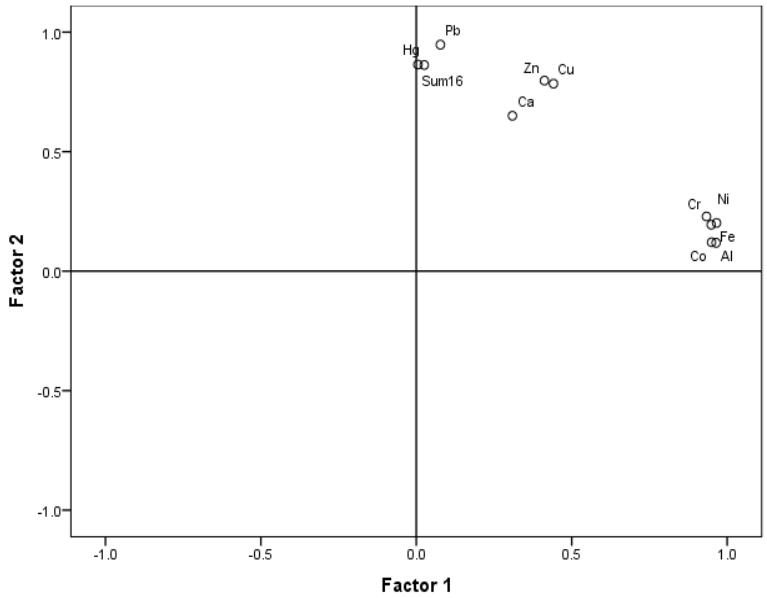

Figure 6.5 - Loading plots of factor 1 and factor 2 of the FA including PAHs and PTEs.

In Figure 6.6 it is shown the spatial distribution of both factor scores, being clear which are the areas most affected by each source. The area with high factor scores of factor 1 is in the southwest and the high levels of Co, Cr and Ni (high loadings on factor 1) were previously related to the basalts from the Lisbon Vulcanic Complex (Cachada et al., 2013). The factor scores of factor 2 have a similar spatial distribution to the ∑16PAHs (Figure 6.3 and Figure 6.6b), with two major hotspots: the airport and the city centre. The reasons for the distribution observed for factor score 2 are the same as referred for PAHs, since the major sources of PAHs and anthropogenic PTEs are common: traffic, railway, shipyard industry, former industry, incineration and crematoriums (Cachada et al., 2013).

In order to understand the role of soil properties on the distribution of PTEs and PAHs, the relationship between them was studied and a new FA was performed. The ∑PAHs was significantly correlated ($\rho<0.01$) with TC, OC, OM and silt (Table 4 of Annex V), yet values were low, being the highest 0.483 and it was observed for TC. Regarding individual compounds the correlations with OC are also low, ranging from 0.298 for anthracene (ANT) to 0.522 for naphthalene (NP). FA that included two general parameters (Table 6.4) considered representative of soil properties (OC and clay) remain similar to the one shown in Table 6.3. Yet it is interesting to note that clay shows a high loading on factor 1 (telluric inputs), whereas OC has similar loadings in both factors, strengthening its role on retention of PTEs (both with telluric or anthropogenic origin) and HOCs. Concluding, both soils properties and specific sources should have influence on the spatial distribution of PTEs and PAHs in Lisbon urban soils.

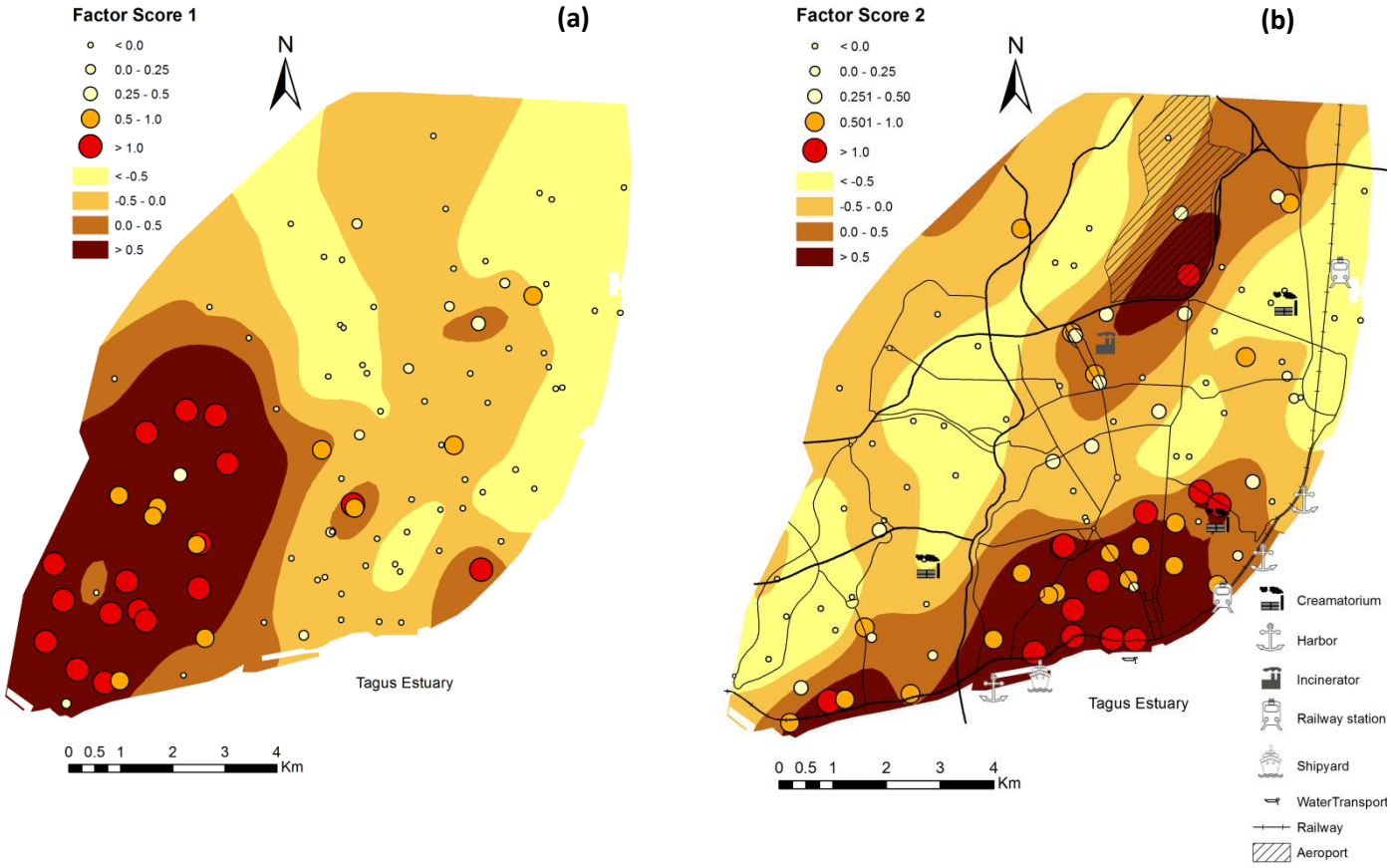

Figure 6.6 - Contour maps (interpolated by ordinary kriging) for the first (a) and second factor (b) factor scores obtained in the FA.

Table 6.4 - Factor loadings of PAHs, PTEs, OC and clay, after Principal Component extraction and Varimax rotation. Coefficients higher than 0.5 are marked in bold. Communalities, eigenvalues and the cumulative variance explained by the factors, are also shown.

	Factor 1	Factor 2	Communalities
Al	**.972**	.103	.956
Fe	**.965**	.186	.967
Co	**.944**	.110	.903
Cr	**.918**	.214	.889
Ni	**.936**	.183	.910
Clay	**.753**	.223	.616
OC	*.500*	*.418*	.424
Ca	.347	**.643**	.533
Cu	*.430*	**.777**	.788
Hg	.018	**.856**	.733
Pb	.088	**.856**	.899
Zn	*.424*	**.795**	.812
Sum16	.022	**.867**	.753
Eigenvalue	7.3	57	-
% Cumulative Expl. Var	2.8	78	-

6.3.3 Risk assessment of PAHs in Lisbon soils

As presented in section 4.3.5, a first approach to assess potential problems concerning contamination of Lisbon soils with PAHs, consisted in compare total levels with generic soil quality guidelines. The percentage of samples that exceeded the Dutch guidelines (Table 2.4) remains unaltered when comparing to the first sampling campaign (section 4.3.5): 21% for the target value and 4% for the maximal value for residential land use (samples 12, 23, 96 and 97). Yet, one of the samples from the airport (sample 96) is slightly above the intervention value from the Dutch guidelines: the level in sample was 39,600 μg kg^{-1}, whereas the intervention value, after correction for the OM content, is 38,600 μg kg^{-1}). In order to obtain a prediction map considering these Dutch guidelines it was necessary to standardize concentrations in Lisbon soils to 10% of OM. Therefore, Figure 6.7 shows a prediction map considering the Dutch guidelines presented in Table 2.4: the target (1000/1,500 μg kg^{-1}), maximum residential (6,800 μg kg^{-1}), and intervention values (40,000 μg kg^{-1}). Observing this figure it is possible to conclude that, with exception of the airport area, levels of PAHs can be considered safe for a residential land use (<6,800 μg kg^{-1}). Yet a considerable area should be subjected to a sustainable soil management since it is considered slightly contaminated (values are between 1,500 and 6,800 μg kg^{-1}).

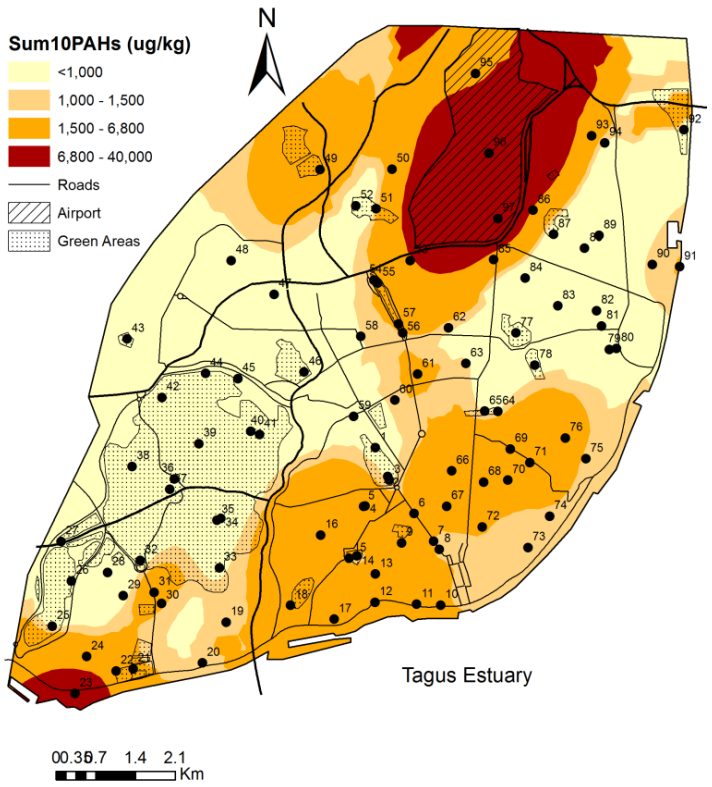

Figure 6.7 - Contour map (interpolated by ordinary kriging) for the ∑10 PAHs corrected to 10% of OM; the class limits correspond to the Dutch guidelines: target (1000/1,500 µg kg^{-1}), maximum residential (6,800 µg kg^{-1}), and intervention values (40,000 µg kg^{-1}).

Similarly, for all other generic guidelines the percentage of samples above the recommended remains unaltered when comparing with the first campaign. Regarding precaution levels from German, 11% of samples (samples 4, 5, 12, 23, 31, 53, 66, 69, 71, 96 and 97) exceeded this value for benzo(a)pyrene (BAP) and ∑16PAHs (Table 2.5), indicating a certain chance of future soil problems. Comparing with the Finish thresholds the values were exceeded in 18% for BAP (samples 9, 11, 16, 17, 22 and 49, in addition to the ones referred for German guidelines) and less than 5% for the other compounds. According to the Finish guidelines a site-specific risk assessment should be carried out in these areas.

6.3.3.1 Potential risks to the environment health (ERA)

The first approach to identify potential risks to the environment was the same as presented in the section 4.3.5.1 and explained with detail in section 2.4. First, the toxic unit (TU), i.e., the ratio between soil concentration and the PNEC value (Eq. 2.2), was calculated for each individual compound (Table 6.5). The PNEC values used in these calculations are the ones presented in Table 2.1, derived by applying an assessment factor to the lowest available NOEC. The toxic units of the

mixture (TUm), i.e. the sum of TU for individual compound, assuming the concentration addition model (CAM), were also calculated and results can be found in Table 6.5. The results of both TU and TUm are similar to the ones obtained when considering the first sampling campaign alone (Table 4.4). However, the maximum values are now much higher than in the first study due to the fact that the second sampling campaign included the hotspots from the airport and from the city centre. For example, considering the TUm, the maximum value rises now to 93. BAP is the compound with higher percentage of samples exceeding the target value of 1, followed by BAA, IND and BGHI. For other compounds the number of samples with TU>1 is lower than 5%. Considering the TUm, in 60% of samples unacceptable effects on organisms are likely to occur as a result of the exposure to the mixture of PAHs.

Table 6.5 - Minimum, median and maximum values of toxic units (TU) for individual compounds and toxic units of the mixture (TUm), using the PNEC values and the MPC (HC5) levels. Percentage of samples with TU and TUm above 1 is also shown.

	PNEC				**MPC (HC5)**			
	Min	Med	Max	TU>1 (%)	Min	Med	Max	TU>1 (%)
NP	0.00	0.00	0.05	0	0.00	0.02	0.33	0
ACY	0.00	0.00	0.25	0	0.00	0.01	0.42	0
ACE	0.00	0.00	1.0	0	0.00	0.00	0.20	0
FLU	0.00	0.00	0.03	0	0.00	0.01	0.17	0
PHE	0.00	0.01	0.49	0	0.00	0.09	3.4	5
ANT	0.00	0.03	1.3	1	0.00	0.02	0.70	0
FLA	0.00	0.03	2.9	2	0.00	0.13	13	8
PYR	0.00	0.04	3.3	3	0.01	0.13	11	8
BAA	0.00	0.28	23	17	0.00	0.03	2.8	2
CRY	0.00	0.05	3.2	3	0.00	0.05	3.0	3
BBF	0.00	0.11	7.5	5	0.00	0.03	2.4	3
BKF	0.00	0.07	5.0	4	0.00	0.02	1.6	1
BAP	0.00	0.51	25	33	0.00	0.03	1.0	0
IND	0.00	0.22	9.4	13	0.00	0.02	0.70	0
DBAH	0.00	0.10	4.8	5	0.00	0.00	0.20	0
BGHI	0.00	0.19	6.1	11	0.00	0.03	1.0	0
TUm	0.03	1.8	93	60	0.03	0.62	42	38

In order to obtain a risk characterization, the TU (Eq. 2.2) were also calculated using the Dutch maximum permissible concentrations (MPC) based on the species sensitivity distributions (SSD) modelling as presented in Table 2.1. Therefore, the TU were now calculated for individual compounds as the ratio between soil concentration and the MPC value, and, as before, the TUm

corresponds to the sum of individual TU. The MPC value used correspond to the HC_5, i.e to the 5th percentile of a SSD based on chronic NOECs. Therefore, as explained in section 2.4, the ratio between the concentration in sample and the MPC value, gives a quantification of the likelihood and a characteristic of the extent of effects. Considering this, the results presented in Table 6.5 corresponds to the percentage of samples for which individual PAHs are at concentrations that will affect a given percentage of species (more than 5%) and whose harms will be similar to the ecotoxicological data used to derive the SSD. When the ratio is equal to 1, it means that the concentration of the compound in the sample is equal to the concentration that has being predicted to affect 5% of the species of the ecosystem. At this level the number of species that is affected by the toxicant is equal to 5% and the risk on adverse effects is equal or lower than 5%. For some samples this ratio was exceeded for individual compounds and considering the TUm the two highest values are 42 and 31 and they were observed in the airport samples, showing that both locals have a PAHs load that will clearly affect a percentage greater than 5% of the species of ecosystem. All other values were below 20, but it should be noted that for almost 40% of samples the probability that a random species is affected by the Σ16PAHs will be greater than 5%.

Comparing results from Table 6.5 it is clear that the PNEC values are much more protective than the HC_5 values, which is mostly based on the fact that the former are based on less information. Moreover, the TU calculated based on the PNEC values tend to increase with the increase on ring number whereas the TU calculated using the HC_5 were higher for 4 ring compounds (FLA and PYR) and this is probably related to the way this values were derived (only the pore water uptake is considered in the second case) as explained in section 2.4.

The calculations of the hazard quotients (HQs), i.e., the ratio between soil levels and a guideline value for individual compounds, and the hazard index (HI), i.e. the sum of HQs (as shown in section 2.4, Eq. 2.1), using the different guidelines for protection of environmental health for Lisbon soils can be found in Table 6.6. Again, these values remain similar to the ones presented in Chapter 4 (Table 4.5). However, the higher number of hotspots when considering the second sampling campaign is reflected in the HQ and HI values. Comparing levels of PAHs observed in Lisbon soils with the quality criteria based on ecotoxicological effects recommended by Danish EPA guidelines presented in Table 2.6, it was observed that 18% of samples exceeded the guideline values for ∑5PAHs (HQ>1) and 28% for BAP. Considering the Spanish guidelines for protection of soil organisms 14% of samples have a HQ>1 for BAP and 8% for FLA. Yet, less than 5% of samples showed concentrations above the Finish and Canadian guidelines for protection of environmental health.

Table 6.6 – Minimum, median and maximum value for the Hazard Quotient (HQ) and Hazard Index (HI) of Lisbon urban soils according to different soil screening guidelines.

	Denmark			**Finland**			**Spanish**			**Canada**		
	Min	Med	Max	Min	Med	Max	Min	Med	Max	Min	Med	Max
NP	-	-	-	0.00	0.00	0.03	0.00	0.02	0.49	0.00	0.01	0.20
FLU	-	-	-	-	-	-	0.00	0.01	0.21	-	-	-
PHE	-	-	-	0.00	0.01	0.52	-	-	-	0.00	0.01	0.95
ANT	-	-	-	0.00	0.00	0.10	-	-	-	0.00	0.00	0.30
FLA	-	-	-	0.00	0.03	2.6	0.00	0.13	13	0.00	0.00	0.37
PYR	-	-	-	-	-	-	-	-	-	0.00	0.01	1.3
BAA	-	-	-	0.00	0.01	1.1	0.00	0.01	0.48	0.00	0.04	5.4
BBF	-	-	-	-	-	-	-	-	-	0.00	0.06	7.6
BKF	-	-	-	0.00	0.01	0.79	-	-	-	0.00	0.03	3.8
BAP	0.00	0.46	45	-	-	-	0.00	0.18	8.7	0.00	0.00	0.23
IND	-	-	-	-	-	-	-	-	-	0.00	0.04	3.5
DBAH	-	-	-	-	-	-	-	-	-	0.00	0.01	0.99
∑PAHs	0.00	0.24	35	0.00	0.03	1.9	-	-	-	-	-	-
HI	-	-	-	0.00	0.07	5.1	0.00	0.34	22	0.00	0.22	25

The HI is higher than 1 in 4% of samples considering the Finish guidelines, 22% for the Spanish and 18% for the Canadian. The samples showing a HI>1 for the most conservative guideline, the Spanish guidelines for protection of soil organisms, where samples 4, 5, 9, 11, 12, 16, 17, 22, 23, 31, 49, 53, 61, 66, 68, 69, 71, 72, 76, 96 and 97.

The calculation of toxic pressure (TP) suggested by the Dutch methodology and explained in section 2.4, was also performed. First, the TP was calculated using the CAM approach as shown in Eq. 2.7, which in this case can be called as multisubstance potentially affected fraction (mSPAF). Two risk limits were used: the MPC that corresponds to a HC_5 and the SRC that corresponds to a HC_{50} (Table 2.1). Using the MPC values the minimum value for mSPAF was 2.6%, the median 38% and the maximum 98%, whereas using SRC the minimum value was 0%, the median 0.70% and the maximum 42%. Figure 6.8 shows the spatial distribution of mSPAF values calculated using the most protective values (the MPC) being possible to identify areas with higher risk. In the two darker areas of Figure 6.8 more than 50% of species are affected by the mixture of toxicants.

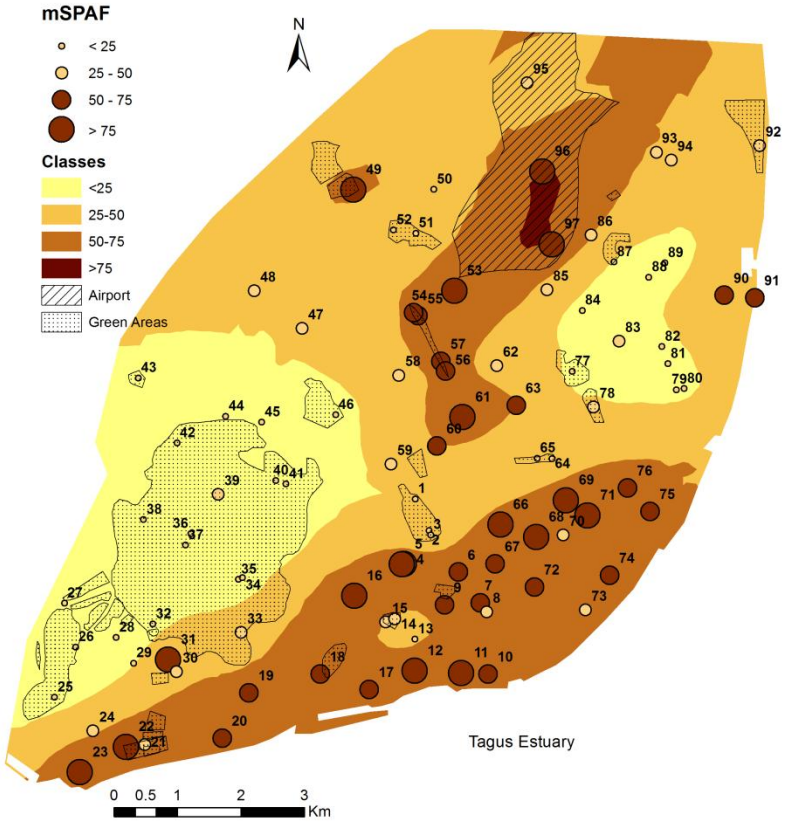

Figure 6.8 - Contour map (interpolated by ordinary kriging) for the mSPAF calculations using the CAM model and the MPC levels (HC_5); the class limits correspond to the 25, 50 and 75% of potential affected species.

Since some authors suggest that toxicity of PAHs would be better represented by the response addition model (RAM), this approach was also considered (de Zwart and Posthuma, 2005; Olmstead and LeBlanc, 2005). Therefore, the PAF_{TMoA} was calculated individually for each compound using Eq. 2.7 and the $mSPAF_{RA}$ was calculated for the mixture of PAHs as shown in Eq. 2.8, and results are shown in Table 6.7. Considering the MPC (HC_5) values, the compounds with the highest median value of PAF were FLA and PYR. Considering the $mSPAF_{RA}$ the median value is 38% and only for 3 samples 100% of species is expected to be affected. Nevertheless, in 36 samples the percentage of species affected is higher than 50%. On the other hand, considering the SRC (HC_{50}) the PAF for individual compounds is very low and the median $mSPAF_{RA}$ is only 0.7%. Moreover, no sample showed a value higher than 50%. These results also show that the mSPAF calculated using the RAM approach is similar to the ones using the CAM

Table 6.7 - Minimum, median and maximum values of PAF for individual PAHs and $mSPAF_{RA}$ for the mixture.

	MPC (HC_5)			**SRC (HC_{50})**		
	Min	Med	Max	Min	Med	Max
NP	0.001	0.011	0.23	0.000	0.000	0.004
ACY	0.000	0.004	0.28	0.000	0.000	0.005
ACE	0.000	0.004	0.16	0.000	0.000	0.002
FLU	0.000	0.004	0.13	0.000	0.000	0.002
PHE	0.003	0.070	0.81	0.000	0.001	0.049
ANT	0.000	0.012	0.41	0.000	0.000	0.008
FLA	0.000	0.098	0.94	0.000	0.001	0.16
PYR	0.005	0.099	0.93	0.000	0.001	0.14
BAA	0.000	0.025	0.75	0.000	0.000	0.035
CRY	0.000	0.037	0.77	0.000	0.000	0.037
BBF	0.000	0.025	0.72	0.000	0.000	0.030
BKF	0.000	0.016	0.62	0.000	0.000	0.019
BAP	0.000	0.022	0.61	0.000	0.000	0.018
IND	0.000	0.012	0.42	0.000	0.000	0.009
DBAH	0.000	0.002	0.12	0.000	0.000	0.002
BGHI	0.000	0.022	0.50	0.000	0.000	0.011
$mSPAF_{RA}$	0.023	0.379	1.000	0.000	0.007	0.423

The TP were also calculated using the HQ approach suggested by Jensen and Mesman (2006), and explained in section 2.4. The environmental quality guidelines from The Netherlands (Table 2.6), Spain (Table 2.7) and Canada (Table 2.8) were used in these calculations. The model, consists

in calculate first the TP of individual compounds (Eq. 2.7) and then, by using the RAM approach, the TP of the mixture (TPm) is calculated as shown in Eq. 2.8.

Considering the MPC values from The Netherlands (derived from assessment factors), the compounds with the highest median TPs were BAP and BAA (Table 6.8) and, with exception of NP, ACE, FLU and PHE, at least one sample presented a TP above 50%. Using the SRC value (derived from assessment factors) the TP of individual compounds are very low and the maximum value was 22% for BGHI in one of the airport samples. These values should be comparable to the ones showed in Table 6.7, since the same method was applied (RAM model using equations Eq. 2.7 and Eq. 2.8). The differences are due to the different methodologies to derive the MPCs and SRCs values, being the pressures calculated based on assessment factors which are much more conservative, especially for the high molecular weight compounds.

Considering the Spanish guidelines for protection of soil organisms (Table 2.7) the higher median value was observed for BAP, with 15 samples showing TPs higher than 50% followed by FLA. Considering the Canadian guidelines for protection of environmental health (Table 2.8), TPs were very low, and only 3 samples showed TP higher than 50% for PYR, BAA, BBF, BKF and IND.

Table 6.8 - Minimum, median and maximum value of toxic pressures (TP) for individual compounds and the toxic pressures of the mixture (TPm) according to different guidelines.

	Dutch MPC			**Dutch SRC**			**Spanish**			**Canadian**		
	Min	Med	Max	Min	Med	Max	Min	Med	Max	Min	Med	Max
NP	0.00	0.01	0.15	0.00	0.00	0.01	0.00	0.02	0.31	0.00	0.00	0.15
ACY	0.00	0.01	0.56	0.00	0.00	0.02	-	-	-	-	-	-
ACE	0.00	0.00	0.12	0.00	0.00	0.00	-	-	-	-	-	-
FLU	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.12	-	-	-
PHE	0.00	0.01	0.41	0.00	0.00	0.02	-	-	-	0.00	0.00	0.49
ANT	0.00	0.03	0.61	0.00	0.00	0.01	-	-	-	0.00	0.00	0.21
FLA	0.00	0.02	0.75	0.00	0.00	0.03	0.00	0.03	0.83	0.00	0.00	0.25
PYR	0.00	0.05	0.86	0.00	0.00	0.14	-	-	-	0.00	0.00	0.58
BAA	0.00	0.24	0.97	0.00	0.00	0.04	0.00	0.00	0.31	0.00	0.03	0.86
CRY	0.00	0.04	0.78	0.00	0.00	0.10	-	-	-	-	-	-
BBF	0.00	0.09	0.90	0.00	0.00	0.08	-	-	-	0.00	0.05	0.90
BKF	0.00	0.05	0.85	0.00	0.00	0.07	-	-	-	0.00	0.02	0.81
BAP	0.00	0.32	0.97	0.00	0.00	0.04	0.00	0.13	0.91	0.00	0.00	0.17
IND	0.00	0.16	0.92	0.00	0.00	0.03	-	-	-	0.00	0.03	0.80
DBAH	0.00	0.07	0.83	0.00	0.00	0.03	-	-	-	0.00	0.01	0.50
BGHI	0.00	0.14	0.88	0.00	0.01	0.22	-	-	-	-	-	-
TPm	0.03	0.77	1.00	0.00	0.01	0.58	0.00	0.19	0.99	0.00	0.15	1.00

The TPm using the Dutch MCP values showed a very high median value (77%), with 16 of the 97 samples showing a toxic pressure of 100%, while for SRC the median value of the TPm is only 1.4% with only one sample showing a value higher than 50% (Table 6.8 and Figure 6.9). Considering the other two guidelines, the median value is lower than 20% in both cases, and 17 samples presented a TPm higher than 50% for the Spanish guidelines and 16 for the Canadian (Table 6.8 and Figure 6.9). Some of these samples with high percentages of affected species were further assessed for their chemical and biological availability as a higher step of risk assessment (Chapter 7and Chapter 8).

From Figure 6.9 it is clear that there is a high variability of protection values, which may depend on the endpoint of protection, but there is also a high variability among countries which may be due to minor differences in the scientific methodology or due to political reasons. Therefore, a harmonization of procedures would be a benefit in terms of risk assessment.

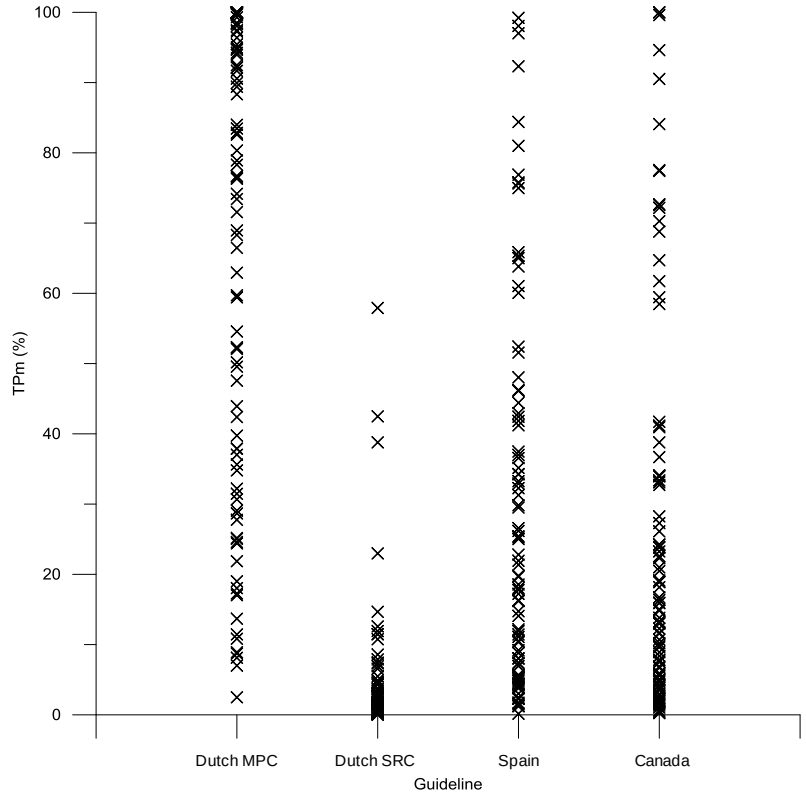

Figure 6.9 – Toxic pressure of the PAHs mixture (TPm) for the 97 samples from Lisbon, considering diferent guidelines values.

6.3.3.2 Potential risks to the human health (HHRA)

As before, the percentage of samples exceeding the guidelines remains similar when comparing to Chapter 4. Regarding the human health protection guidelines, only less than 5% of samples showed levels above the recommended, with exception of the ∑5PAHs (15% considering the Danish soil quality criteria) and BaP (31% for Danish guidelines, 10% for Canadian and 15% for Dutch).

The PAH toxic potency of soil samples, expressed as BAP-equivalents (BAPeq) was calculated as described in section 2.5.2. In Figure 6.10a it is possible to observe the distribution of BAPeq concentrations, based on its statistical distribution: minimum (0.59 µg BAPeq kg^{-1}) quartiles (23, 74 and 203 µg BAPeq kg^{-1}), the upper outlier limit (468 µg BAPeq kg^{-1}) and the maximum value (7,653 µg BAPeq kg^{-}). It is interesting to note that the distribution map is very similar to the one of the distribution of the ∑16 PAHs (Figure 6.3). The mean value for the BAPeq in Lisbon was 347 µg BAPeq kg^{-1}), and comparing with other cities around the world, these levels are lower than the ones found in Shangai (428 µg BAPeq kg^{-1}) but higher than levels found in Beijing (181 µg BAPeq kg^{-1}) and in Tarragona (124 µg BAPeq kg^{-1}) (Jiang et al., 2009; Liu et al., 2010a; Nadal et al., 2004).

Even that only the Canadian guidelines refer to the use of BAPeq, it was decided to compare the distribution of the ∑BAPeq with all the guidelines values of BAP. Therefore, Figure 6.10b shows the distribution of ∑BAPeq in which the intervals chosen were based on the several guidelines available: 15 µg kg^{-1} is the USEPA soil screening level for residential areas; 100 µg kg^{-1} corresponds to the Italian value for residential areas and to the Danish soil quality criteria; 200 µg kg^{-1} corresponds to the Spanish value for residential areas; 600 µg kg^{-1} is the Canadian limit for protection of human health considering a total lifetime cancer risk (TLCR) of 10^{-6}; 1000 µg kg^{-1} corresponds to the Danish cut-off criteria; 2000 µg kg^{-1} corresponds to the German value for playgrounds and to the Finish lower guideline value considering a TLCR of 10^{-5}; 4000 µg kg^{-1}corresponds to the German value for residential areas; and 5,300 µg kg^{-1} is the Canadian limit for protection of human health considering a TLCR of 10^{-5}. No sample reached the value of 10,000 µg kg^{-1} that is the maximum for commercial areas according to the Italian guidelines and the German limit for parks and recreational areas. Only in the white areas of the map in Figure 6.10b, the levels were below any of the guidelines and in the light yellow area values were above the conservative value from USEPA.

In Figure 6.10b it are also identified some of the playgrounds and schools sampled. By crossing this information it is possible to identify areas in which a more detailed assessment should be made, since these are sensitive land uses regarding the human exposition.

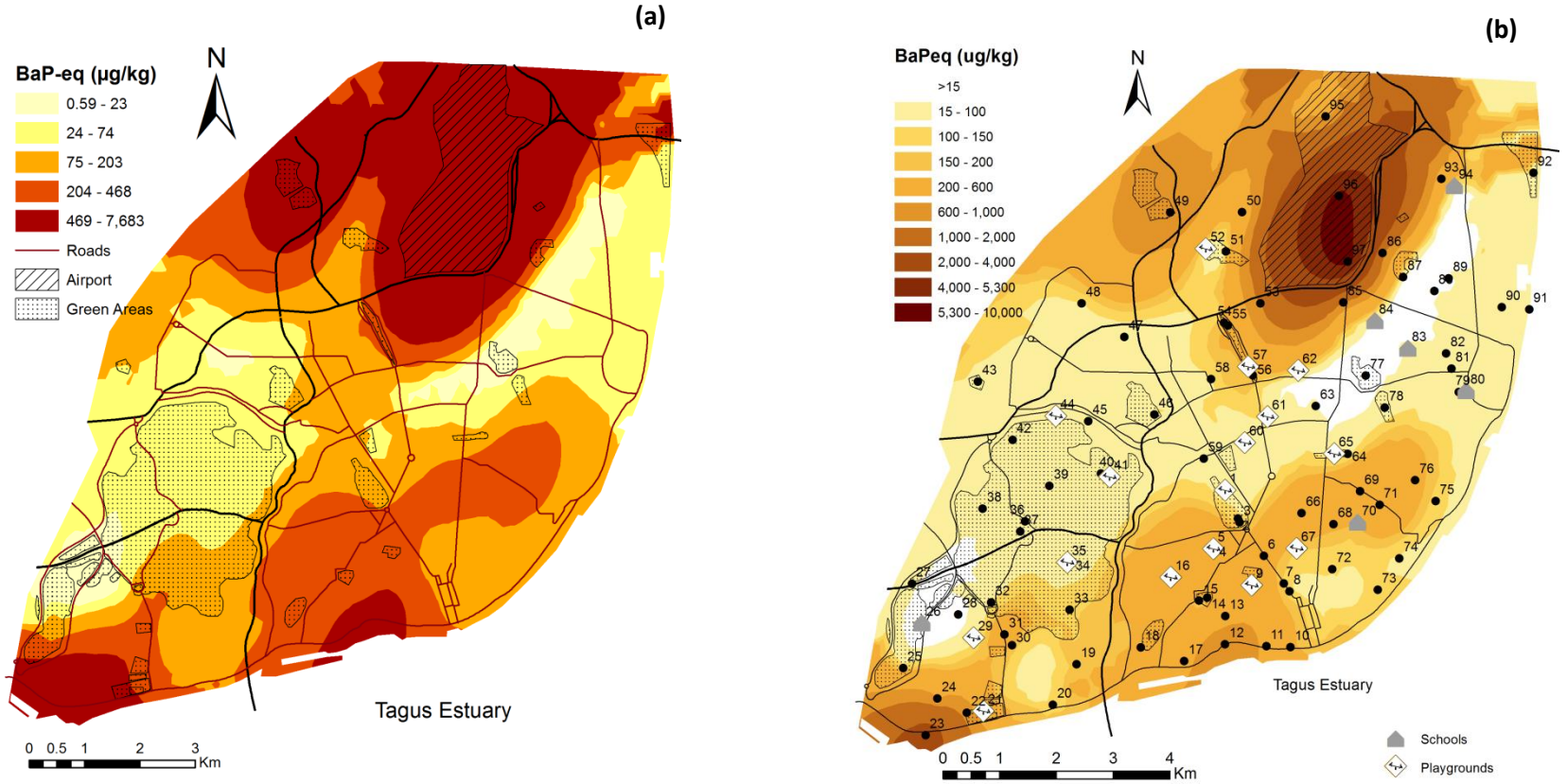

Figure 6.10 – Contour map for ∑BAPeq levels in Lisbon area, which was interpolated by ordinary kriging; the class limits correspond to the minimum, the quartiles (25, 50 and 75), the upper outlier limit, and the maximum value (a), and considering the different guideline values for human health protection (b).

The non-cancer hazard quotient, calculated according to USEPA methodology, was below the target value of 1 for all contaminants which have an RfD_O (NP, ACE, FLU, ANT, FLA and PYR). Similarly, using the Dutch methodology as described in section 2.5.1 the HQs were always below 1.

Regarding cancer risk assessment, the results for residential, worker and recreational exposure to PAHs are shown in Table 6.9. Risks for a residential exposure are high, being the reasonable maximum exposure, given by the upper confidence level of the mean (UCL 95%), higher than 10^{-6} for cancer risk and higher than 10^{-5} for mutagenic risks. In both cases the values were higher than the target excess individual lifetime risk, which is one-in-one-million (10^{-6}) (USEPA, 2011b). Comparing with other studies, Lisbon showed slightly higher total risks for BAPeq than the ones found in Tarragona or in Beijing (for normal conditions) (Nadal et al., 2011; Peng et al., 2011a).

Figure 6.11 shows the prediction map of the TLCR for a residential land use, being possible to identify the areas which may represent a potential risk. The most contaminated areas in Lisbon represent a risk of 1.2E-04 and are located nearby the airport and at lower extent in the southwest. In Figure 5 of Annex V it is possible to cross this information about probable risks with the population density of the Lisbon districts and conclude that in some of the most populated areas the risks are between 10^{-6} and 10^{-5}. If samples from the airport are excluded, the cancer risk given by the UCL (95%) drops to 6.5E-06 and for mutagenic risks to 2.5E-05, but still higher than the target value. Considering other ingestion rates (50 mg for adult and 100 mg for children) as suggested the Dutch methodology (Table 2.2) the risks decrease but the UCL (95%) is still higher than 10^{-6}: 5.8E-06 with a median value of 7.7E-07 and a maximum value of 8.0E-05.

Table 6.9 - Statistical data of total cancer and mutagenic risks for different land uses of Lisbon soils.

	Cancer risk			**Mutagenic risk**	
	Residential	Worker	Recreational	Residential	Recreational
UCL	8.97E-06	2.38E-06	5.06E-07	3.33E-05	2.13E-06
median	1.19E-06	3.16E-07	7.54E-08	4.66E-06	3.17E-07
mean	5.59E-06	1.48E-06	2.39E-07	2.19E-05	1.01E-06
90%	9.88E-06	2.62E-06	6.71E-07	3.99E-05	2.82E-06
min	9.50E-09	2.52E-09	7.07E-10	3.53E-08	2.97E-09
máx	1.24E-04	3.28E-05	4.13E-06	4.59E-04	1.74E-05

The cancer risks were calculated for occupational land use (outside workers, e.g. gardeners) and it was observed that PAHs levels in Lisbon may represent some concern (Table 6.9), being the UCL (95%) higher than 10^{-6}. In recreational areas, cancer and mutagenic risks were also calculated with the most contaminated site showing a value higher than 10^{-6}, but the UCL (95%) was lower than the target value. However, mutagenic risks are higher, with the most contaminated site representing a risk higher than 10^{-5}, whereas the UCL (95%) is higher than 10^{-6}.

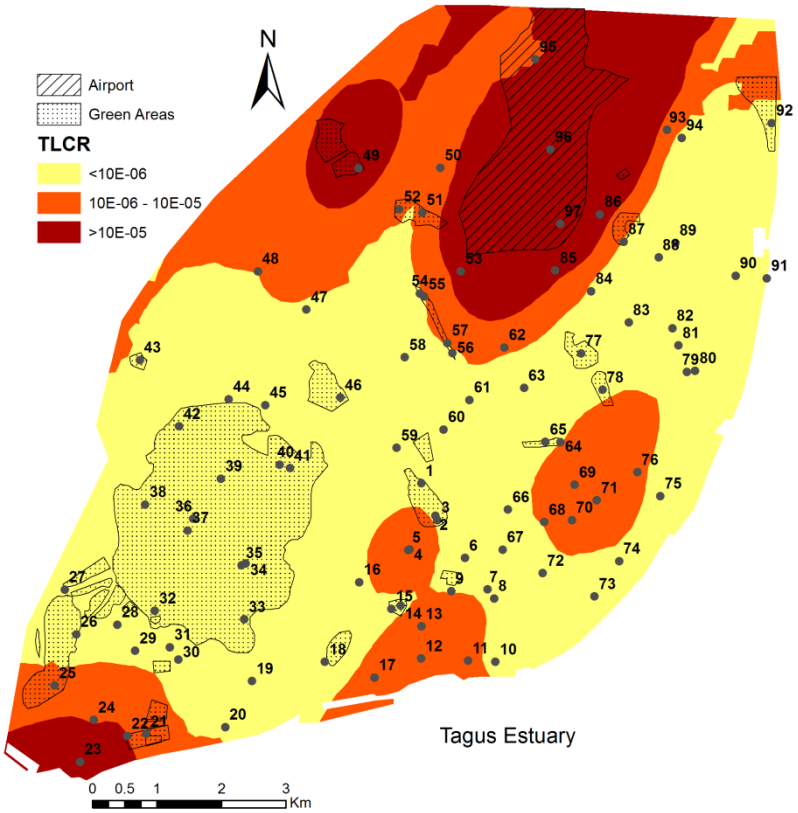

Figure 6.11 - Contour map interpolated by ordinary kriging for the cancer risks considering a residential land use; the class limits correspond to the risk limits suggested by different guidelines.

Chapter 7

APPLICATION OF A SOLID PHASE EXTRACTION METHOD TO ASSESS THE MOBILITY OF PAHS IN URBAN SOILS

7.1 INTRODUCTION

As shown in previous chapters, Lisbon urban soils may be severely affected by PAHs contamination. Since some PAHs are known or suspected of carcinogenic or mutagenic properties, the fate of these compounds in the soil is critical. Traditional analytical methods of organic pollutants determination in soils consist of vigorous extractions with organic solvents, giving an indication of the total concentration. However, it is now known that this total amount should not be directly related to environmental risk since only a fraction can be available for partitioning between soil and soil solution and thus uptaken or transformed by organisms or leached to groundwater (Semple et al., 2003). Therefore, the effects of contaminants to organisms and to the environment are related to its potential available fraction, rather than its total concentration. Once in soils, HOCs can undergo time-dependent sequestration (or irreversible sorption) resulting in a diminution of the available fraction, whereas the total concentrations can remain similar. This phenomenon is especially important in urban soils, where contamination is a result of a long-term accumulation (as shown in previous chapters) and it is not easy to know the age of contamination.

Traditional methods can, therefore, give an overestimation of risks and, in order to overcome this, several chemical methods to predict the available fraction (chemical availability) have been developed as discussed in Chapter 3. The solid phase extraction using the hydrophobic polymer Tenax-TA© as adsorbent is a depletive water based non-exhaustive extraction method to estimate the bioaccessible fraction and gives the actually plus the potential available concentration. This method has been used for determining the size of the rapidly desorbing fraction as well as the slow desorption fraction. Yet, the most used approach is the estimation of the rapidly desorbing fraction, i.e., the sorbed concentration which can desorb towards the pore water within a short time. Therefore, 6- or 24h extractions are used, based on the principle that the fraction that can be desorbed to soil solution within a few hours is the most important when assessing availability (Cornelissen et al., 2001). The advantages of using this resin were already addressed in section 3.2.1, and include for example the easy separation of the resin from the soil solution, and its re-use. Even that Tenax extractions have been successfully used for predicting PAHs bioavailability in sediments, for soil samples the number of studies is scarce and results are not consistent. Considering the findings reviewed in Chapter 3, regarding biodegradation, it was concluded that the fraction resistant to desorption could be an indication of the fraction resistant to biodegradation, however, the first tend to be higher. When comparing Tenax extraction with earthworm and plant accumulation, results are not clear. Nevertheless, this rapidly desorbing

fraction can be useful to assess the PAHs mobility, and therefore the potential for leaching. Yet, few studies have applied this method to estimate the available fraction of field contaminated samples. Due to the importance of studying the fate of PAHs in the soils, in this study a water based extraction, using the Tenax resin as adsorbent, was applied in order to evaluate the mobility and availability, and estimate the risks of contamination in Lisbon urban soils. Moreover, the influence of soil characteristics and PAHs properties regulating contaminants fate in soils was also studied.

7.2 MATERIALS AND METHODS

7.2.1 Tenax Extractions

Ten soil samples (Figure 7.1) from Lisbon urban area were selected to study the mobility of PAHs, based on their levels and soil properties. Tenax extractions were performed by preparing a soil suspension with ultra-pure water using a ratio of 1:10 (m/v), and agitated during 6h at 60 rpm in the presence of Tenax-TA© (60/80 mesh) (Cofield et al., 2008; Gomez-Eyles et al., 2010; Li et al., 2005). The extractions were performed at room temperature and protected from sunlight. The amount of Tenax added was 3 times the content of organic matter (OM) of soil (MacRae and Hall, 1998).

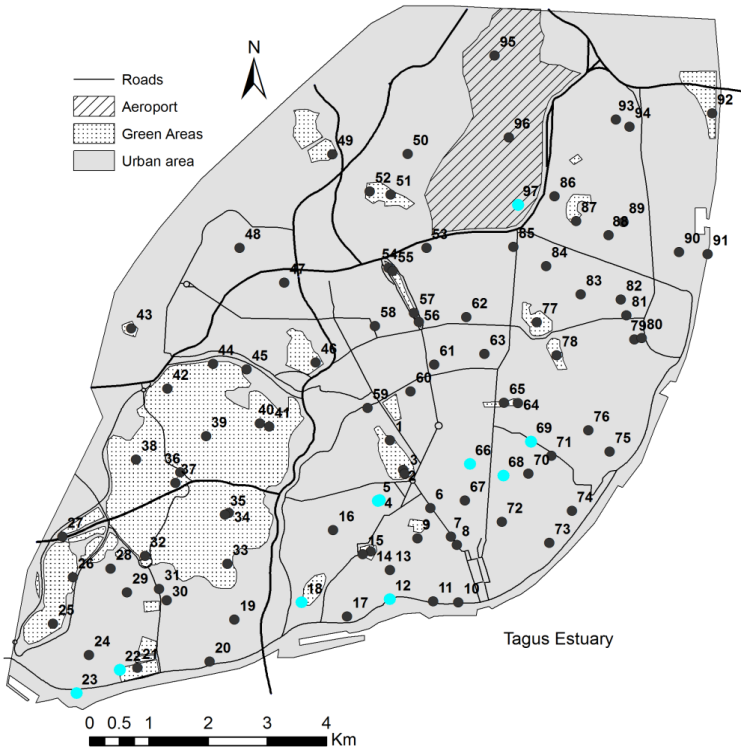

Figure 7.1 - Samples selected for studying the mobility of PAHs (blue dots).

After separating the Tenax from the soil solution, PAHs were extracted, first with a mixture of hexane and acetone (1:1) and then with hexane (Cofield et al., 2008; Gomez-Eyles et al., 2010). Extracts were cleaned up and analysed as described in previous chapters to obtain the water soluble concentrations of soils. The internal standards (mixture of deuterated PAHs) were added in the first extraction of Tenax. Before use, Tenax was prepared by washing (3 times for each solvent) with water, acetone, a mixture of hexane:acetone (1:1) and finally hexane (1:10 m/v). The resin was then dried in an oven at 75°C. Quality control included the analysis of Tenax before its use, the extraction of blanks and spiked samples.

7.3 RESULTS AND DISCUSSION

7.3.1 Method validation

The proportion of 3 times more Tenax TA per gram of soil than the OM present in sample was selected since it has been suggested to be enough in order to not saturate Tenax (MacRae and Hall, 1998). Yet, a proportion of 1:1 (m/m) was also tested and no differences were observed (the variability observed when comparing samples extracted with both methods was <20%). The analysis of blanks (ultra-pure water) using the maximum mass of Tenax used in the samples (3 g) revealed that levels of PAHs were below the limit of detection (LOD) for all compounds except of naphthalene (NP) and in few cases for phenanthrene (PHE). In order to evaluate the recoveries of the method, water spiked with three different levels of PAHs was tested, using different amounts of Tenax (0.2 and 1.5g). Results (Table 7.1) indicate that the method is accurate and precise for all compounds with exception of NP, which showed very high recovery percentages, especially for the lowest spike level, being related to the signal observed in blanks. Phenanthrene in the lowest spiked level also showed a recovery somewhat high, which could be also related with the signal observed in blanks. For this reason it was decided to test the Tenax for interferences always before it use. Only for NP the problems remained and for this reason it was decided to exclude it from the study.

Recovery tests were also performed using soil samples. Two samples with very low levels of total PAHs, one with 15% of OM and another with 3.0%, were spiked with 5 and 15 μg kg^{-1} of each individual compound in soil. This allowed checking the absence of interferences and if the amount of Tenax used was enough to adsorb all PAHs. Since recoveries obtained were between 70 and 116% it was possible to assume that the method could be applied to soil samples, with different levels of OM.

Table 7.1 – Tenax extraction recoveries from spiked water samples at three different concentrations of PAHs, with the mean value, standard deviation (STD) and the relative standard deviation (RSD).

	5 ng			10 ng			50 ng		
	Mean	STD	RSD (%)	Mean	STD	RSD (%)	Mean	STD	RSD (%)
NP	203	42	21	180	28	16	115	4	4
ACY	100	4	4	66	5	8	79	6	8
ACE	74	8	11	85	8	9	96	6	6
FLU	94	7	8	102	7	7	104	4	4
PHE	120	7	6	96	7	7	90	5	6
ANT	83	12	15	79	7	9	85	4	4
FLA	86	8	9	88	6	7	86	5	5
PYR	88	6	7	88	7	8	86	5	5
BAA	82	7	9	99	7	7	89	7	7
CRY	84	10	12	77	6	8	78	3	4
BBF	86	9	10	85	8	9	83	7	9
BKF	88	5	6	87	10	12	87	7	8
BAP	68	7	10	78	7	9	83	5	6
IND	88	13	14	82	10	12	84	5	6
DBAH	91	13	14	85	8	9	87	9	10
BGHI	80	5	7	81	6	7	85	6	7

Table 7.2 - Tenax extraction recoveries from soil samples spiked at two different concentrations of PAHs, with the mean value, standard deviation (STD) and the relative standard deviation (RSD).

	5 µg kg^{-1}			**15 µg kg^{-1}**		
	Mean	STD	RSD (%)	Mean	STD	RSD (%)
ACY	108	12	11	109	6	6
ACE	86	6	7	72	4	6
FLU	98	7	7	95	8	8
PHE	97	11	11	106	7	6
ANT	87	8	9	92	5	6
FLA	88	9	10	86	8	9
PYR	93	11	12	89	7	8
BAA	87	11	13	91	3	4
CRY	89	8	9	96	10	10
BBF	91	9	10	85	10	12
BKF	116	3	3	104	8	8
BAP	84	8	9	70	3	4
IND	76	4	5	99	10	10
DBAH	80	6	8	86	2	3
BGHI	79	17	22	91	3	3

7.3.2 Water soluble fraction

The 10 samples chosen have different soil properties and PAHs concentrations, as shown in Table 7.3. Eight of the samples showed very high levels of PAHs and they correspond to samples identified as outliers (Figure 6.2), and for the other two the levels are lower but still above the Netherlands target value (Figure 6.7). All samples selected, with exception of samples 18 and 68, were identified as representing a potential risk to the environment (Figure 6.8) and to human health (Figure 6.10). The majority of samples are from the city centre (only sample 97 is from the airport), which has a higher population density and thus the impacts on human health can be significant. In addition, the sites selected are parks or gardens where it is important to maintain ecosystem functions and soil quality.

As can be observed in Table 7.3, the water soluble concentrations are very low, especially when comparing with total extractions. With exception of PHE, the 3 ring compounds, together with dibenzo(ah)anthracene (DBAH), were not detected in soil extracts (Table 1 of Annex VI). This behaviour could be related with the low total concentrations of these PAHs in soil samples as described in previous chapters. Looking for example to Figure 6.4, it can be observed that these compounds are the ones showing the lowest median percentages. Hence, the further discussion will be based only in the sum of the other 10 PAHs: PHE, fluoranthene (FLA), pyrene (PYR), benzo(a)anthracene (BAA), chrysene (CRY), benzo(b)fluoranthene (BBF), benzo(k)fluoranthene (BKF), benzo(a)pyrene (BAP), indeno(1,2,3-cd)pyrene (IND), and benzo(ghi)perylene (BGHI).

Table 7.3 - Resume of total and water soluble concentrations of PAHs (∑15) in selected samples and their general properties.

Sample	Total PAHs ($\mu g\ kg^{-1}$)	Soluble PAHs ($\mu g\ kg^{-1}$)	%OM	%OC	pH	%Sand	%Silt	%Clay
4	3,849	26.1	5.7	2.5	7.30	51	40	9.3
5	3,413	5.21	7.7	2.8	6.43	60	31	9.0
12	11,939	22.4	8.5	3.5	6.92	59	33	8.4
18	1,226	4.42	5.1	2.6	7.16	63	27	9.2
22	6,073	15.6	12	4.2	7.24	71	16	13
23	22,105	41.9	8.3	3.2	7.25	65	21	14
66	4,298	7.91	5.3	2.3	6.94	59	32	9
68	1,224	5.23	2.9	1.5	7.30	56	31	13
69	4,161	5.71	4.2	1.9	6.71	64	25	11
97	73,381	228	18	7.4	6.71	78	16	7.3

The available fraction of PAHs, i.e., the ratio between the water soluble and the total concentrations (∑10PAHs), was lower than 1% in all samples (Figure 7.2a). These values indicate

that PAHs are probably retained in soils and thus presenting low mobility and low potential for leaching to groundwater or to be uptaken by organisms through soil solution.

As referred previously, the high levels of PAHs in Lisbon soils should be a result of long term accumulation due to diffuse pollution. One of the reasons that lead to this conclusion was the high levels found in old gardens and parks from the city centre (Figure 6.3). The samples chosen to assess the water soluble fraction were some of the most contaminated ones and they were all taken from old parks and gardens from the city centre (with exception of the one from the airport). Therefore, the low mobility of PAHs in selected samples support the referred hypothesis, since the low available fraction indicates that PAH suffered sequestration after prolonged residence times in soil (aging) (Li et al., 2007). The concentration of available PAHs is generally related to the total concentration as shown in Figure 7.2a. However, the lower available fractions were found for the most contaminated samples, reinforcing the idea that the high levels of PAHs found in the most contaminated sites are mostly related to the accumulation and aging of contaminants. Likewise, the three higher available fractions correspond to the lowest total concentrations.

Several other studies have used non-exhaustive extractions to estimate the chemical available or bioaccessible fraction of PAH in field contaminated soils as referred in section 3.2.1. As explained, different methods will give different results becoming difficult to make comparisons. Despite that, other studies report similar available percentages to the ones found for Lisbon urban soils. For example in a gas work site the available percentages were: 0.004% using water extraction, 0.2% using 1% methanol, and 5% using 50% methanol (Bergknut et al., 2007). In roadside soils, using cyclodextrin extraction, the available fraction ranged between 1 and 5% and in this case the authors related the low values with the presence of soot (Johnsen et al., 2006). On the other hand Krauss et al. (2000) report available percentages of PAHs in urban and peri-urban soils ranging from 6 to 68% but in this case the extractant was a 50% methanol solution. Actually, non-exaustive extractions with mild solvent solutions extract a much higher fraction of contaminants comparing with the water soluble and therefore it is expected that mild solvent extractions will potentially overestimate the availability of contaminants. From these examples it is also important to highlight the differences in the nature of OM.

In the previous chapters it was found that the total amount of PAHs can be related to the content of organic carbon (OC) present in soils, indicating that it can be an important factor on the retention of PAHs in soils. Figure 7.2b shows the relationship between total PAHs levels normalized to OC versus the concentration of available PAHs also normalized to OC content.

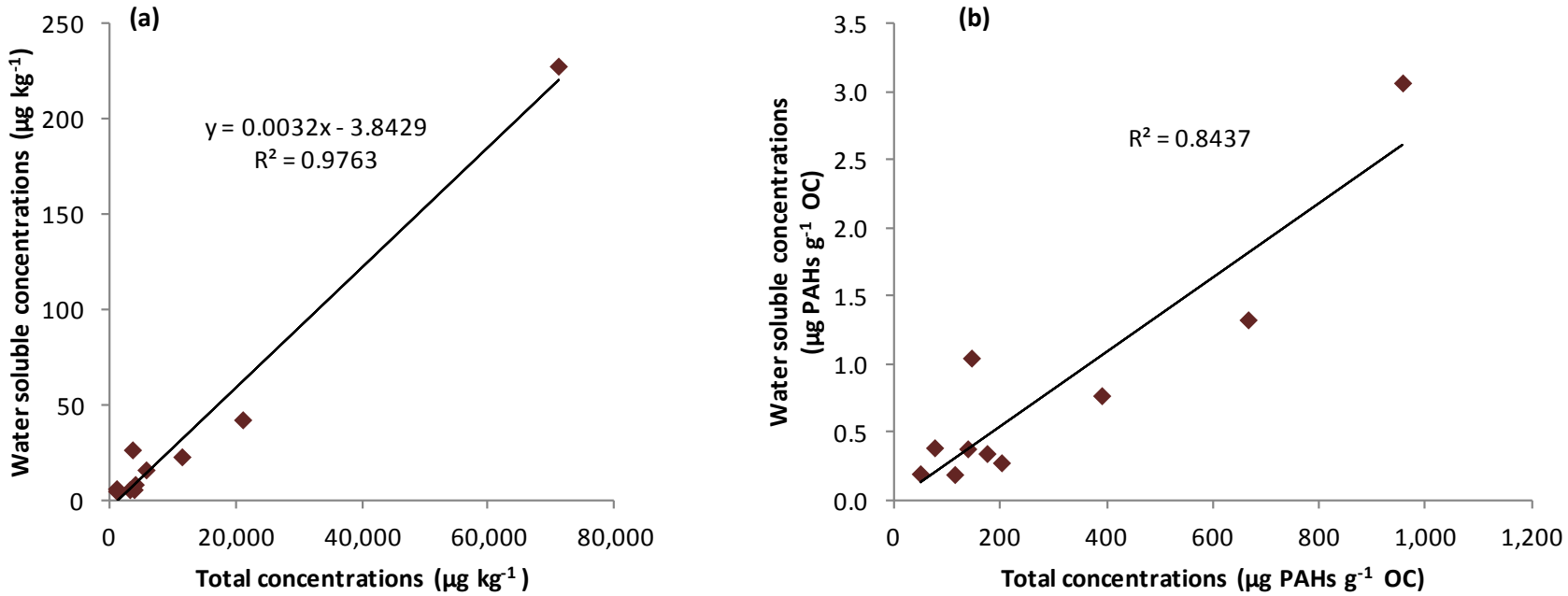

Figure 7.2 - Relationship between total and water soluble concentrations (a) and between total concentrations and water soluble concentrations, both OC normalized (b).

The R^2 is lower when normalizing to the OC content, indicating that both soil properties and aging of contaminants may have an influence on mobility of PAHs in soils.

The influence of soil properties in the chemical available fraction of PAHs (given by non-exhaustive extractions) from genuinely contaminated soils is not clear (Bogan et al., 2005; Hawthorne et al., 2002). In the present study no significant correlations between the water soluble fraction and soil properties were observed, and this behaviour was also observed by other authors (Braida et al., 2004; Čvančarová et al., 2013; Hawthorne et al., 2002). Yet, the rate constant of rapid desorption of PYR has been shown to be inversely dependent of OM content in spiked soils aged for 120 days, but no relationship was observed for the desorption percentage (Li et al., 2007). On the other hand, unlike what would be expected, on manufactured gas plant (MGP) soils a poor positive correlation for the ∑15 PAHs and OC content was observed (Bogan et al., 2005). Indeed, the quality of soil's OM has been pointed as an important factor controlling desorption (Gomez-Eyles et al., 2012). Figure 7.3 shows the relationship between the available percentage for the Σ10PAHs and the OC content of soil samples. Although no clear trend is observed, it seems that for samples with the lowest OC content the variability of results is higher. Moreover these samples with an OC around 2% are also the samples with the lowest levels of PAHs, again indicating that aging may play an important role and the OC content may be a key factor on retaining PAHs in soils. Even so, in order to obtain more revealing conclusions it would be necessary to have more data, especially for samples with OC content between 3.5 and 8%.

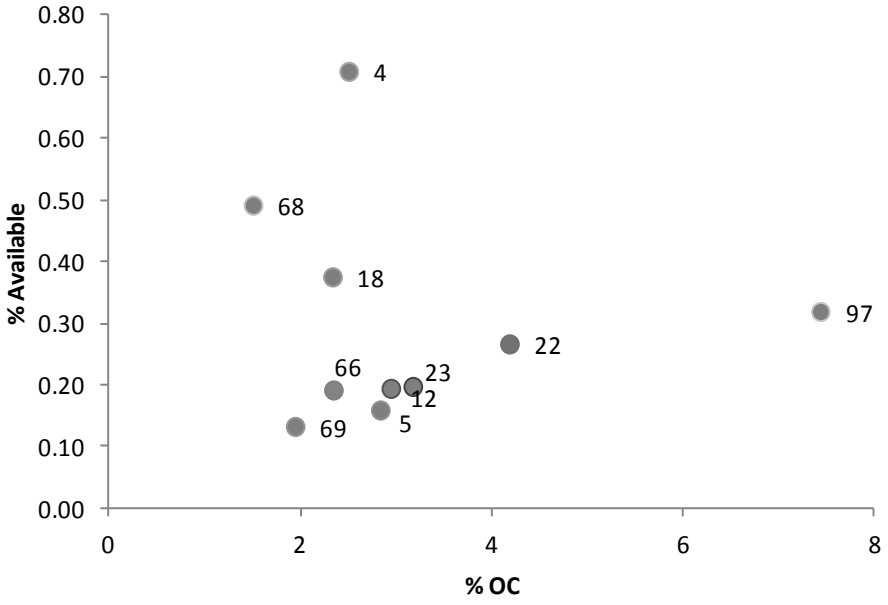

Figure 7.3 - Relationship between the percentage of PAHs available (∑10) and the OC content of soil samples from Lisbon urban area.

7.3.3 The PAHs profiles, compound and soil properties

Profiles of PAHs obtained by the total extractions were different from the ones of the water extractions (Figure 7.4). It was observed that PAHs with ≤4-rings show a higher percentage in the water extractions, whereas higher molecular weight PAHs (≥5-rings) are more abundant in total extractions. Indeed, the soluble fraction (mean value) decreases as the number of rings increases: 0.5% for 3 ring, 0.3 for 4-ring and 0.2 for 5+6-ring PAHs. This behaviour meets the expected, i.e. non-exhaustive extractions reflect individual PAH properties. In spite of being normally very abundant in naturally contaminated soils, PAHs with 5+6-ring tend to have lower extractabilities due to their recalcitrant nature and higher tendency to associate with OM (Bogan et al., 2005; Hawthorne and Grabanski, 2000). In addition, these compounds have higher octanol-water partition coefficient (K_{OW}) and soil-water partition coefficients (K_{OC}) values and lower water solubility (Table 1 of Annex II), and therefore they are less prone to be transferred to soil solution. On the other hand, the higher available percentages of compounds with lower molecular weight PAHs is probably related to their higher water solubility and lower K_{OW} and K_{OC} (Table 1 of Annex II).

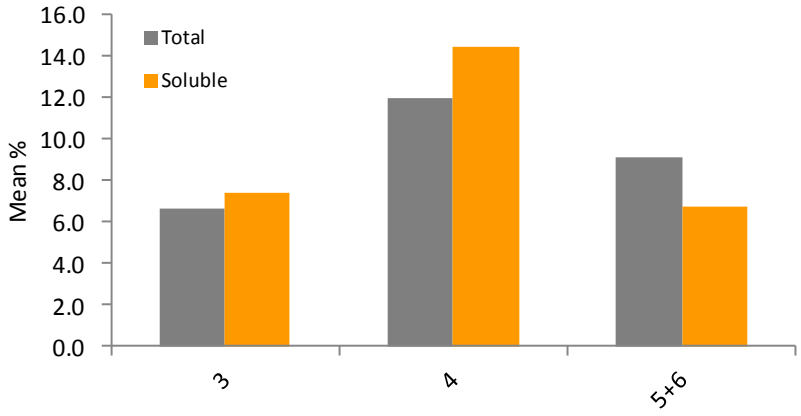

Figure 7.4 - Profiles for total and water soluble extractions of Lisbon urban soils, by group of PAHs (3, 4 and 5+6 ring compounds).

Problems in assessing the available fraction of PAHs with 5 and 6 rings using water based extractions have been reported in literature, mostly due to their low desorption rates and low solubility. For example, Hawthorne et al. (2001) did not calculate the F_{rap} of these PAHs in MGP soils using XAD-2 during 120d, because the release was too slow to observe a two phase behaviour. Other studies showed that Tenax or XAD-4 desorption percentages (at 120 days or 15

days, respectively) from MGP contaminated soils decrease with ring size, being negligible for PAHs with higher molecular weight (Bogan et al., 2005; Li et al., 2005). A very low desorption percentage (6%) was also observed in coke oven soils in which the profiles were dominated by high molecular weight PAHs, after 210 d of extraction with Tenax (Ahn et al., 2005). For desorbed compounds, the fraction also depended on the ring size, being observed values of 58, 3.3 and 1.1% for NP, PHE, and FLA, respectively (Bogan et al., 2005).

The relationship between available fraction and the K_{OW} of the 10 PAHs detected in the water based extractions from all samples is shown in Figure 7.5. It seems that there is a tendency for compounds with higher K_{OW} to show lower available fractions. However, CRY which is a 4 ring compound and has a log K_{OW} of 5.81 shows an anomalous behaviour since the available percentages are in general higher than for FLA and PYR (K_{OW} of 5.18 and 4.96, respectively). In some samples, BBF and BKF (K_{OW} of 6.12 and 6.11, respectively) also showed an anomalous behaviour by presenting relatively high available percentages.

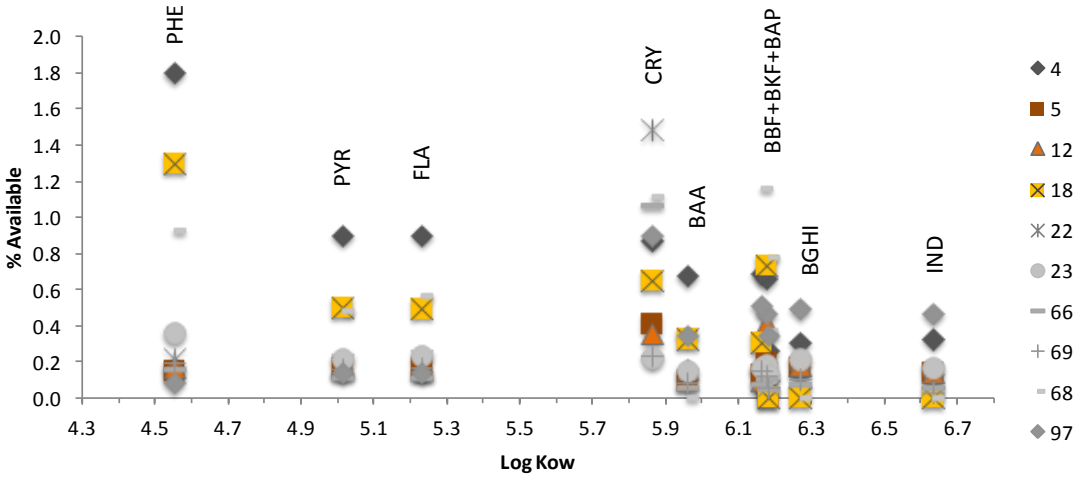

Figure 7.5 - Variation on the percentage of individual PAHs available in each soil sample according to their log K_{OW}.

In Figure 7.6a it is possible to observe the relationship between the mean available fraction and the K_{OC} value of individual compounds. As observed in Figure 7.5 for K_{OW}, there is a decrease in the available percentage with the increase in K_{OC} value. In this figure it is much clear the behaviour of CRY (K_{OC}=5.6), which shows the highest mean available percentage. On the other hand BAA (K_{OC}=5.7), which is an isomer of CRY, showed one of the lowest available fractions. If

excluding CRY and the benzofluoranthenes (K_{OC}=6.1), the tendency to decrease the available fraction with the increase in K_{OC} value is much clear (Figure 7.6b).

The reason for the anomalous behaviour of CRY could be due to the co-elution of another PAH, the triphenylene. These two compounds are isomers and they cannot be separated with the chromatographic conditions used, and therefore the results obtained concern the sum of the two compounds. In spite of being isomers, the solubility and K_{OW} of the two compounds are different, which could explain the results. Whereas CRY has a K_{OW} of 5.81, triphenylene has a K_{OW} of 5.49. Similarly, the water solubility of triphenylene is higher than CRY, and consequently if the former compound is present in a sample, the availability will be greater than if only CRY is present. However, triphenylene is not normally considered when analysing PAHs in urban soil samples and therefore there is no information about its contribution the total PAH burden.

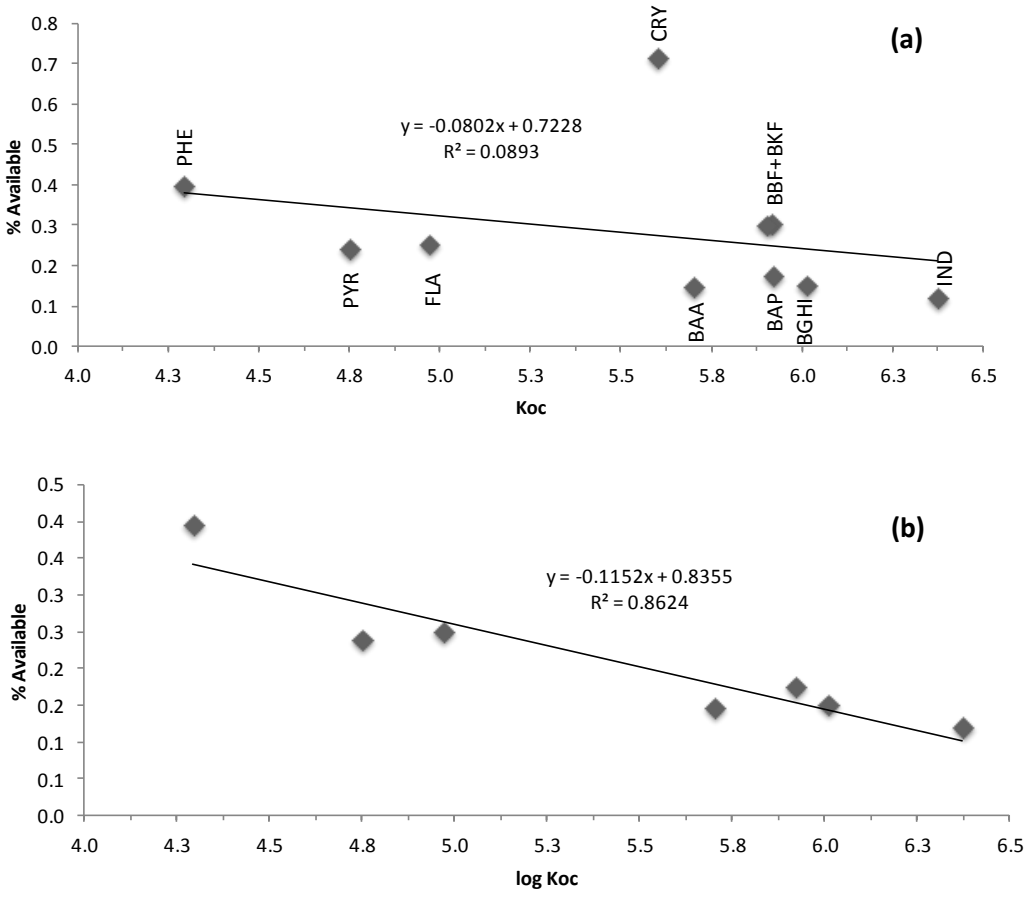

Figure 7.6 - Variation on the mean percentage of available PAHs in the soil samples according to log K_{OC}, with (a) and without (b) CRY, BBF and BKF.

Regarding benzofluoranthenes, there is a similar problem of co-elution of the isomer benzo(j)fluoranthene together with BKF. As before, the physic-chemical properties of the two isomers are different; this could explain some of the anomalous results. In addition, the isomer BBF and BKF are not completely separated in the chromatogram, and thus the integration of chromatographic peaks can be a major issue. When dealing with such small amounts of these compounds the errors resulting from data analysis can have a strong influence.

When looking to the behaviour of each samples in Figure 7.5, it's possible to conclude that there is a high variability of results, with some samples showing the expected behaviour whereas for others it's difficult to relate the availability with compound's properties. There are several reasons to explain this: a) the origin of contamination; b) the age of contamination; c) soil properties.

Regarding the influence of soil properties, as explained before, little is known and results from the literature are not conclusive. In the present study no clear relationship was observed between the ∑10PAHs and the OC content (Figure 7.3). Also when looking to available percentages of individual compounds it is difficult to reach any conclusion (Figure 7.7). In both cases (3+4-rings and 5+6-rings) it appears to occur a decrease in the available percentage with the increase of OC content, and, as for the total amount, the variability of results seems to be higher for samples with less than 2.5% of OC. Even that for some compounds such as PHE the available fraction seems to decrease with the increase of OC, there is a high variability of results. If looking to PAHs with 5 and 6 rings (Figure 7.7b) it also appears that there is a global tendency to decrease the available fraction as the OC increases, but again the variability is high. Once more, there is a lack of samples with OC above 3.5% which make these observations little supported. In addition, it should be noted the anomalous behaviour of CRY, especially for the two samples with the highest OC content (sample 22 and 97) (Figure 7.7a). For samples with an OC content lower than 4% it appears that the available percentage of CRY tend to decreases with the increase in OC, but with a high variability of results. It can also observed in Figure 7.7a that the available percentage of CRY is higher than for other compounds with lower K_{OW} and solubilities as shown in Figure 7.5, with exception of PHE in some of the samples. For the other compounds the behaviour is the expected in most of the samples: for 3+4-ring compounds the % available decreases by the following order PHE>FLA>PYR>BAA.

The sample with the highest content of OC (sample 97) is actually the most contaminated one and it is located in the airport. Therefore, its location can explain the available fraction higher than expected according with the soil properties of the sample, especially the 5 and 6 ring

compounds, since there is a continuous input of contaminants and may reflect that it is a result of a more recent contamination.

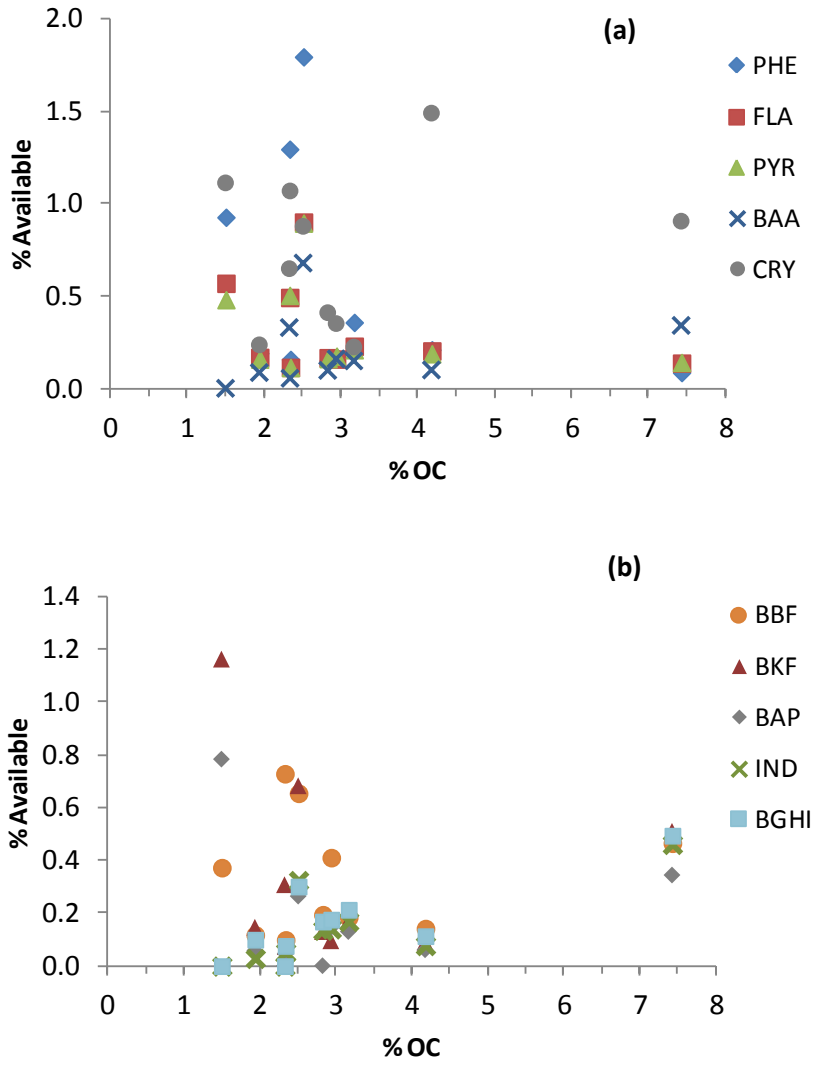

Figure 7.7 - Relationship between the available percentage of PAHs with 3 and 4 rings (a) or 5 and 6 rings (b) and the OC content of soil samples.

7.3.4 Risk assessment implications

With exception of samples 18 and 66, all other samples were previously identified as requiring a more detailed risk assessment. Loibner et al. (2006) suggest an approach for the inclusion of chemical availability data in risk assessment which consists in the use of bioaccessible data in the refined screening phase (Tier 2), by directly comparing the concentrations obtained in the non-

exhaustive extractions with soil screening levels. This approach is based on the fact that soil screening levels are obtained using freshly spiked soils and therefore no aging is likely to occur. Considering the water soluble concentrations instead of total levels, the values were much below the guidelines, with the HQ<1 in all cases. Only when considering the Spanish guidelines for protection of aquatic organisms, the HI is above 1 for sample 97. When calculating the TU and the TUm, results are similar, being always below 1. Therefore, in view of this approach levels of PAHs in Lisbon urban soils could be considered safe since they are not likely to pose a risk.

The water soluble concentrations were also used to recalculate the cancer and mutagenic risks for protection of human health. The HQ was not calculated because they were always below 1 when using total extractions. Considering the most sensitive land use (residential) no sample is likely to pose a risk since the values were always below 10^{-6}. Considering the mutagenic risk the levels are also below 10^{-6} for all samples expect sample 97 which shows a risk of 1.52E-06.

Results from this availability study shows that the potentially available fraction of PAHs in Lisbon urban soils is very low and with this limited leaching the potential risks to the environment and to human health are not as high as derived from the assessment based in total concentrations.

Chapter 8

BIOAVAILABILITY OF PAHS IN URBAN SOILS

8.1 INTRODUCTION

As discussed in Chapter 3, the suitability of chemical methods to predict bioavailability of PAHs in field contaminated soils was not yet demonstrated. Up to now, bioassays are considered the most accurate approach to assess the bioavailable fraction, since they integrate all the interactions between contaminants, matrix and organism. Direct methods such as the accumulation in organism's tissues can be very useful when mixtures of contaminants are present, as in the case of urban soils. Earthworms (Lumbricidae) are regarded as a model organism and they are normally used to estimate the potential exposure of soil biota. As explained in Chapter 3, the main reasons for their use are: earthworm's uptake of contaminants directly from soils; they show a high degree of pollutant accumulation; and they are of easy handling and easy to culture under laboratorial conditions. Ecologically they are of extreme importance due to soil services where they are directly involved and they are considered indicator species (Lavelle et al., 2006; Pulleman et al., 2012). Further, they live in intimate contact with soil and they are likely to reflect the quality of this matrix (Pulleman et al., 2012).

The traditional approach of risk assessment (RA) consists in the application of the equilibrium partition theory (EqPT) in order to estimate potential risks to organisms, as described in sections 3.2.2 and 3.3.2. Briefly, the aim of EqPT is to predict the concentration of a contaminant in an organism based on levels found in soils. According to this theory, the chemical partitioning between organisms' lipids and soil organic carbon (OC) is based on thermodynamic principles, resulting in constant accumulation factors which represent chemical distribution between lipid and organic carbon at steady state (Leppänen and Kukkonen, 2006). Indeed, the EqPT has been successfully applied to derive ecological screening benchmarks for PAHs in sediment invertebrates; however the applicability of this theory to terrestrial organisms can be questioned and there are some criticisms (Sijm et al, 2000; Bergknut et al, 2007). In spite of taking into account that sorption reduces availability, it does not include desorption phases and the way compounds are sorbed in soils. As a consequence, many studies reported that biota-to-soil accumulation factor (BSAF) measured were lower than predicted by this model (Jager et al., 2003; 2007), whereas others reported overestimations (Jonker et al., 2007; Kreitinger et al., 2007) and others found that it was applicable (Krauss et al., 2000). As explained previously (section 3.2.2), the deviations to EqPT can be due not only to physico-chemical factors but also to the species related differences. One of the assumptions of EqPT is that the uptake of HOCs by organisms occurs via passive diffusion from soil solution through epidermis, leaving out that earthworms can also access contaminants from solid phase, through gut uptake. Other factors that may cause a

deviation in EqPT estimations are biotransformation, active excretion and reduction of body burden due to reproduction.

In order to overcome some of the drawbacks of the traditional approach, especially the ones related to the physico-chemical factors, it has been suggested an improvement in the EqPT, by including the chemical available fraction as a more relevant factor for bioavailability (van der Wal et al., 2004). Therefore, this improvement is based either on the use of freely dissolved pore-water concentrations (e.g. SPME fibers) or/and on the rapidly desorbed fraction (e.g. Tenax extraction) to estimate body residues of HOCs (Ten Hulscher et al., 2003; van der Wal et al., 2004). However, as discussed in Chapter 3, there is no clear evidence that this approach could improve the prediction of body residue levels.

In spite of the growing number of studies that have been made, there is still a lack of knowledge on bioavailability of field contaminated soils, and especially of urban soils. Therefore, this work aims at assess the bioavailability of PAHs in some selected urban soils from Lisbon and investigate factors affecting it. Since the application of EqPT in soils is not well documented, it was also intended to verify its application as a simple approach in RA. The usefulness of rapid desorbed fraction to improve the EqPT was also considered.

8.2 EXPERIMENTAL PROCEDURE

8.2.1 Preparation of soils for bioaccumulation experiments

Before starting with bioaccumulation assays in the urban soils the methodology was tested using spiked soils. Therefore, for spiking experiments an artificial soil was used according to the OECD guidelines for the testing of chemicals (OECD, 2010). The artificial soil was obtained by mixing kaolinite clay, quartz sand and peat. For a soil with 5% content of organic matter (OM_{dw}) the proportions of each fraction were: 20% kaolonite, 75% sand and 5% peat. Four days before starting the experiment the water content of soils was adjusted to 40% of its maximum water holding capacity (WHC). Soil pH was tested at beginning of experiment to assure that it was within the required range (6.0±0.5).

The soil water content, the soil pH and the OC content of soils were determined as explained in section 4.2.1. The determination of the maximum WHC was performed according to the method ISO 11269. First, soil samples were placed in plastic flasks, with the bottom replaced by filter paper, and immersed in water for 3 h. Then, samples were placed for about 2 h on

absorbent paper to reject the excess of water, and finally the maximum WHC was determined by weighing samples before and after drying at 105°C until weight stabilization.

A spiking solution containing a mixture of the 16 PAHs was prepared in acetone with a final concentration of 300 µg mL^{-1} of each compound. A portion of soil (25 g) was weighted to a glass amber flask and the spiking solution (1.25 mL) was added and left in a fume hood for one night to evaporate the acetone. After that, another 225g of soil were weighted to the flasks, homogenised in an agitator and allowed to equilibrate during four days. Finally the content of all flasks were mixed together and homogenised before starting the bioaccumulation experiments. For control soils the procedure was the same, by adding the same volume of acetone as in spiked soils. Spiked and control soils were tested for PAH concentrations and homogeneity by analysing 5 replicates of each using the same analytical procedures as described in section 5.2.1 for dust samples. Yet, due to the high content of water in these soils, sodium sulphate was added before the beginning of the extractions.

Urban soil samples selected for the bioaccumulation assays (Figure 8.1) were chosen in order to have a large range of concentration of PAHs and other properties; however, due the high amount of soil needed to conduct the experiment the selection was limited to the availability of soil mass.

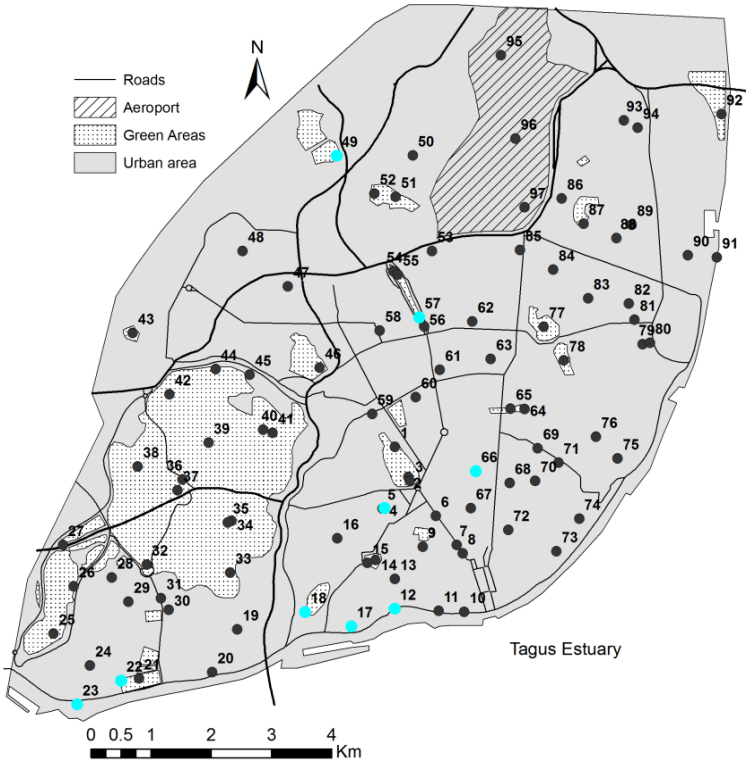

Figure 8.1 - Selected samples for the study of the bioavailability of PAHs in Lisbon urban soils (blue dots).

Samples were sieved (<2mm) and the moisture content was adjusted to 40% of the maximum WHC. As control samples on the urban soils bioaccumulation assays it were used artificial soils (prepared as described above) with three different contents of OM (5, 8 and 10%).

8.2.2 Earthworms bioaccumulation assays

Bioaccumulation assays were performed according to the OECD guidelines (OECD, 2010). Earthworms (*Eiseinia andrei*, Lumbricidae) used in the assay came from a permanent laboratory culture maintained at the Department of Biology, University of Porto. Only adult animals with clitellum were used, and the mean weight of individuals was 358±58 mg. Individuals were acclimatized for 72h in an artificial soil with similar OM content of test soils. Five individuals were transferred to an aluminium box containing 250g of soil, with 4-5 cm height in order to allow the burrowing behaviour of organisms, and a food ration was added. The food consisted in horse manure, sieved to 2 mm and decontaminated by subjecting it to 2 cycles of cold (freezing) and hot (oven at 100°C). For each control, spiked sample or urban soils, 3 replicates were tested for bioaccumulation. Boxes were kept at 20±2°C with a light cycle of 16/8h light/dark (400-800 lx) during 21 days. The moisture was kept constant by regular weighing the test containers, and food was added once a week. At the end of the experiment worms were separated from soil, rinsed with water and allowed to purge overnight on moist filter paper. After that, individuals were weighted and immediately frozen (-18°C).

8.2.3 Earthworm extraction and analysis

Dry weight was determined after freeze-drying using three replicates of a sub-sample of test worms with 5 individuals each. The lipid content was determined using the procedure described by Smedes (1999), which consists in a gravimetric determination after an extraction with 2-propanol and cyclohexane. The proportion of 1g tissue (fresh weight, fw) to 1.6 mL of 2-propanol and 2 mL cyclohexane was maintained.

Two saponification procedures were tested for the extraction of PAHs from approximately 1.5g (fw) of earthworms exposed to spiked soils. The first method included the following steps as described by Gomez-Eyles et al. (2012): firstly, earthworms were grounded with sodium sulphate in a weight proportion of 1:7 (m/m), and then 10 mL of KOH 0.5 M were added followed by 10 mL of a mixture of hexane:acetone (1:1); secondly, the mixture was sonicated for one hour (beginning at 45°C, agitated each 15 min); thirdly, 5 mL of water were added and agitated for ten minutes; finally, the organic phase was allowed to separate and 5mL of hexane were again added to the sample and agitated for 10 min (this last step was repeated twice).

The second method tested consisted in a methanolic saponification with 30 mL KOH 1M during 3h at 80°C, which has been implemented in our lab to extract PAHs from bivalve samples. The extract is then submitted to a liquid-liquid extraction with 10 mL hexane (10 min agitation), and this last step is repeated twice.

Cleanup of extracts was performed using SPE cartridges with 1.5 g of alumina (5% deactivated) and 1.5 g of silica (3% deactivated). Sorbents were first washed with 10 mL of hexane:DCM (9:1) and conditioning was performed with 10 mL of hexane. Elution was then performed with 20 mL of hexane:DCM (9:1) and the volume of the extracts was reduced in a rotary evaporator, transferred to a vial and further reduced to 0.2 mL using a gentle stream of pure nitrogen.

Both procedures were tested by analysing spiked samples at different levels and for the second method the extraction efficiency was also tested by analysing a reference material (SRM2977 – Mussel tissue). The internal standard (a mixture of 5 deuterated PAHs) was added to the samples before extraction and an injection standard (mixture of 2 deuterated PAHs) was added to the extract before analysis. The detection limits of the method ranged between 1.6 and 10 µg kg^{-1}, depending on the compound and the mass of sample. Analytical backgrounds were also determined in control individuals.

The BSAF value (in kg_{OC}/kg_{lip}) in each sample was determined as explained in section 3.2.2, using Eq. 8.1, where: C_{worm} is the concentration of PAHs in earthworm tissue (µg kg^{-1} fw), f_{OC} is the fraction of organic carbon in soil (kg kg^{-1}), C_{soil} is the concentration of PAHs in soil (µg kg^{-1}) at t21 (Jager et al., 2003) and f_{lip} is the fraction of lipid in earthworm tissues (kg kg^{-1}, fw).

Eq. 8.1

$$BSAF = \frac{(C_{worm} \times f_{OC})}{(C_{soil} \times f_{lip})}$$

8.3 RESULTS AND DISCUSSION

8.3.1 Comparison of the extraction procedures

The recoveries of the internal standards ranged between 60 and 111% for the first method and between 50 and 112% for the second method. Regarding spiked samples, results of both saponification methods were also comparable ranging between 72 to 113% in the first case and between 80 and 112% in the second method. Samples from the bioaccumulation assay with spiked soils were also analysed by both methods and no statistical differences were observed between the two methods. Since the methanolic saponification has been already proved to be an efficient extraction methodology by the analysis of a certified reference material (results were

within the certified value; the 95% confidence interval of the recoveries of the certified values was 109+10%) it was decided to use this method in further studies.

8.3.2 Accumulation of PAHs in earthworms using spiked soils

Concentrations of PAHs in soil samples at the beginning of experiment are shown in Table 8.1, and the homogeneity was verified by analysing 5 replicates of the spiked soil before the experiment, being the relative standard deviation (RSD) always less than 15% for individual compounds. The water content in soil samples was 14.2±0.5% and the real OC content was 1.86±0.28%. The levels of some compounds found in soil control samples were considered negligible since they are lower than 2 μg kg^{-1} as shown in Table 8.1. At the beginning of the experiment (t0) the expected levels of PAHs in soils were around 2000 μg kg^{-1}, and with exception of naphthalene (NP), acenaphthylene (ACY), and acenaphthene (ACE), results were in accordance with this value (recoveries higher than 80%). The low concentration observed for the referred compounds are probably related to the higher fugacity of the compounds that resulted in losses due to volatilization during the spiking procedure, since the soil samples were left in a fume hood overnight to evaporate the acetone.

Table 8.1 - Concentrations (μg kg^{-1}) of the individual compounds in control and spiked soils (dw), at the beginning (t0) and at the end of experiment (t21), and in earthworms (fw). Biota-to-soil accumulation factors (BSAF, kg_{OC}/kg_{lip}) are also shown.

	Soils			Earthworms		BSAF
Compound	Control	SPK t0	SPK t21	Control	SPK (t21)	
NP	0.61	21.8	-	-	-	-
ACY	bdl	861	26.0	bdl	13.2	0.53
ACE	bdl	1184	59.1	bdl	48.4	0.85
FLU	bdl	1483	56.5	bdl	27.6	0.50
PHE	1.3	1669	142	4.2	89.4	0.65
ANT	bdl	1710	324	bdl	448	1.43
FLA	1.5	1970	1505	3.7	3493	2.40
PYR	1.5	1967	1781	5.0	4107	2.38
BAA	bdl	1937	2082	bdl	4539	2.25
CRY	1.3	1927	2046	bdl	4980	2.52
BBF	1.3	1952	2175	bdl	2880	1.37
BKF	1.0	2026	2194	bdl	4125	1.94
BAP	bdl	1767	1855	bdl	2800	1.56
IND	1.1	2063	2201	bdl	1524	0.72
DBAH	bdl	2159	2211	bdl	3230	1.51
BGHI	1.3	2078	2083	bdl	826	0.41
∑PAHs	10.9	25,892	20,713	20.0	33,118	1.65

bdl=below detection limits

Due to the low levels of NP observed at the beginning of the experiment this compound was not included in the further discussion of results. At the end of the experiment all compounds with 3 rings showed levels at least 80% lower than observed at the beginning of the experiment, being especially interesting the difference observed between the isomers phenanthrene (PHE) and anthracene (ANT). These results are in accordance with previous studies: for example, Gomez-Eyles at al. (2011) reported significant losses of 2 and 3-ring compounds in freshly spiked samples during the 20 days of exposure. These losses can be related either to volatilization of these compounds or to degradation due to microbial and earthworm activity. In the particular case of PHE and ANT, the differences between the two isomers could be due to higher losses by volatilization of the former, since it has a higher vapour pressure (Table 1 of Annex II). The analysis of control samples without earthworms would be helpful to understand if losses were due to their activity. In order to exclude the hypothesis of microbial activity soils should have been sterilized. For fluoranthene (FLA) and pyrene (PYR) the decreases in concentrations were 24 and 10% respectively, whereas for all other compounds no differences were observed.

Regarding the earthworms, no mortality was observed and the mean mass loss was 20%, which was the limit indicated in the OECD guidelines for validation of the test. The water content of worms was estimated as 83.7±0.5%, and the lipid content as 1.8±0.3%. The highest concentrations of PAHs in earthworm tissues were observed for the 4 ring compounds (mean value of 4,280 μg kg^{-1}), whereas the lowest concentrations were observed for the 3 ring compounds (mean value of 126 μg kg^{-1}). This behaviour could be, in addition to losses in soil samples due to both volatilization and biodegradation as referred, because the organisms were able to metabolize them. Again, it is interesting to highlight the difference in concentrations between the isomers PHE and ANT, which is in accordance to the decrease of PHE in soils at the end of the experiment. Indeed, previous studies reported accumulated percentages of PHE in aged soils as small as 3.3% by *E. fetida* (Kelsey and Alexander, 1997) and as high as 13.7% for ANT (Tang and Alexander, 1999). Another study states that some organisms are able to biotransform and eliminate PHE (You et al., 2006).

The BSAF values for the spiked sample can be found in Table 8.1 and they ranged from 0.41 for BGHI to 2.52 (kg_{OC}/kg_{lip}) for CRY, being the value for the ∑15PAHs of 1.65. These values are much lower than the ones found by Jager et al. (2000), which obtained BSAFs values for *E. andrei* of 7.3 (kg_{OC}/kg_{lip}) for PHE, 8.2 for FLA, 3.9 for PYR, in a freshly spiked soil with various PAHs concentrations. Kreitinger et al. (2007) used deuterated PAHs to calculate BSAFs for *E. fetida* and they report values closer to the ones found in the present study for compounds with 4 and 5 rings

(ranging from 1.16 to 5.14). However, for the low molecular weight (LMW) compounds the values obtained by Kreitinger et al. (2007) were much lower (always below 0.5) than the ones from our study and this difference may be related to the soil data used in calculations. Due to losses of LMW PAHs in soils during the experiment, the BSAF values can be very different when using levels occurring at t0 or t21. In the present study the levels at the end of the experiment were considered since the steady state was achieved, but when t0 values in the soil are considered the BSAF values are always below 0.1. In addition, the calculation of BSAF values has other uncertainties sources such as in the calculation of lipid content of tissues and in the OC/OM contents. For example, Van der Wall et al. (2004) determined a lipid content of 2.3% for *E. andrei* but other authors use an estimated value of 1%. Some authors used the OM content instead of the OC while other authors used an estimation of the OC based on the OM content (OC=58%OM). Therefore, the BSAF values should be interpreted and compared with caution.

8.3.3 Accumulation of PAHs in earthworm using urban soils

The characterization of selected soils for the bioaccumulation tests is shown in Table 8.2. Levels of individual PAHs and of the ∑PAHs found in the selected soil samples and in the exposed earthworms are shown in Table 8.3. In this experiment 100% mortality was observed in the three replicates of sample 66, but for other samples no mortality was observed. The reason for the mortality in sample 66 could be attributed to the presence of other contaminants or to other characteristics of samples. However, levels of PTEs analysed and general parameters were similar to other samples as can be observed in Table 8.2 and Table 1 of Annex VII, and therefore there is no apparent reason for the mortality observed.

Table 8.2 - Characterization of soil samples used in the bioaccumulation assay.

Sample	∑16PAHs (µg kg^{-1})	%OM	%OC	pH	%Sand	%Silt	%Clay
4	3,849	5.7	2.5	7.30	51	40	9.3
12	12,581	7.1	3.5	6.92	59	33	8.4
17	2,475	8.5	3.0	6.82	58	34	8.3
18	1,208	5.1	2.6	7.16	63	27	9.2
22	6,073	12	4.2	7.24	71	16	13
23	22,105	8.3	3.2	7.25	65	21	14
49	7,108	14	4.0	6.74	72	32	5.4
57	1,719	8.8	2.5	6.67	55	31	14
66	4,298	5.3	2.3	6.94	59	32	9.0

Table 8.3 - Individual PAHs concentrations (µg kg^{-1}) in selected Lisbon urban soil samples (dw) and earthworms (fw).

	4		**12**		**17**		**18**		**22**		**23**		**49**		**57**	
	Soil	worm	Soil	worm	Soil	worm	Soil	worm	Soil	worm	Soil	worm	Soil	worm	Soil	worm
ACY	5.6	bdl	35.5	bdl	2.5	bdl	3.0	bdl	25.5	bdl	178	bdl	15.9	bdl	12.5	bdl
ACE	12.2	bdl	22.0	bdl	5.3	bdl	7.2	bdl	17.9	bdl	31.1	bdl	13.7	bdl	3.3	bdl
FLU	12.1	bdl	25.8	bdl	5.4	bdl	7.7	bdl	14.4	bdl	33.6	bdl	16.0	bdl	5.8	bdl
PHE	262	4.9	582	7.3	111	14.0	80.5	8.2	415	4.6	866	18.8	226	5.4	95.5	5.5
ANT	48.1	bdl	96.2	bdl	20.7	bdl	21.8	bdl	60.0	bdl	240	bdl	65.2	bdl	32.6	bdl
FLA	510	9.2	2,262	17.8	314	31.1	196	17.7	991	9.5	3,295	65.7	1086	20.4	268	10.8
PYR	451	12.7	2,254	22.6	268	31.9	177	17.9	916	10.3	3,172	72.6	1034	21.6	224	10.0
BAA	310	7.6	809	13.1	162	16.3	96.0	7.1	442	6.3	1,611	34.4	632	16.6	131	6.9
CRY	316	13.0	829	18.4	199	25.5	99.7	9.3	541	9.6	1,834	44.7	650	23.0	166	10.0
BBF	481	10.1	1,034	17.2	370	24.7	107	9.2	589	8.5	2,803	43.9	791	19.4	181	8.4
BKF	186	6.6	845	13.4	181	20.6	84.2	6.8	468	6.0	1,296	29.7	628	17.5	124	8.7
BAP	416	8.6	1,253	15.8	235	18.1	113	8.7	549	7.4	2,166	43.7	770	18.3	154	7.5
IND	384	7.2	1,132	13.6	264	17.7	89.1	6.5	498	6.6	2,143	41.1	524	10.8	137	6.1
DBAH	87.5	bdl	190	bdl	47.0	bdl	31.7	bdl	115	bdl	463	bdl	161	bdl	38.0	bdl
BGHI	366	7.8	1,213	15.2	289	19.1	94.8	7.0	430	7.3	1,974	46.1	494	10.7	145	7.3
∑15PAHs	3,849	88	12,581	154	2,475	219	1,208	98	6,073	76	22,105	441	7,108	164	1,719	81

bdl=below detection limit

Overall, the mean mass loss of the earthworms was 9%, much below the 20% indicated in the OECD guidelines for validation of the test. The humidity of worms was 81.0±0.5%, and the lipid content was 1.7±0.2%.

Levels of PAHs found in earthworms are low, especially when compared with levels observed in soil samples (Table 8.3), with the accumulated percentage of PAHs (tissue:soil ratio) ranging between 1.3% (sample 12) and 9.2% (sample 17). Even so, these values are higher than the ones observed in previous studies for *E. fetida* in manufactured gas plant (MGP) soils (∑22PAHs): 0.04% of the total observed by Bergknut et al. (2007) and an average value of 0.06% observed by Kreitinger et al. (2007).

Once the 3-ring compounds (except PHE) and dibenzo(ah)anthracene (DBAH) were not detected in earthworm tissues (Table 8.3), they were excluded from the following discussion, being the total levels now referred to the sum of 10 PAHs (∑10PAHs). The non-detection of 3 ring compounds and DBAH, with exception of PHE (Table 8.3), is certainly related to the low levels present in soil samples, and in the case of LMW PAHs volatilization and degradation may also play a role as referred previously. Indeed, the profiles of PAHs in earthworm residues were dominated by 4 ring compounds (mean value of 12%) followed by the 5+6 ring compounds (7.6%) and PHE, the only 3 ring compound showing detected levels (mean value of 1.1%). This trend is very similar to the one observed for total extractions of soils (Figure 8.2): 11, 7.9 and 1.5% for 4, 5+6 and 3-ring compounds, respectively. Similarly, in MGP soils, only FLA, PYR, BaA and CRY contained detected levels in earthworms (*E. fetida* and *L. terrestris*) among 12 PAHs analysed (Parrish et al., 2006).

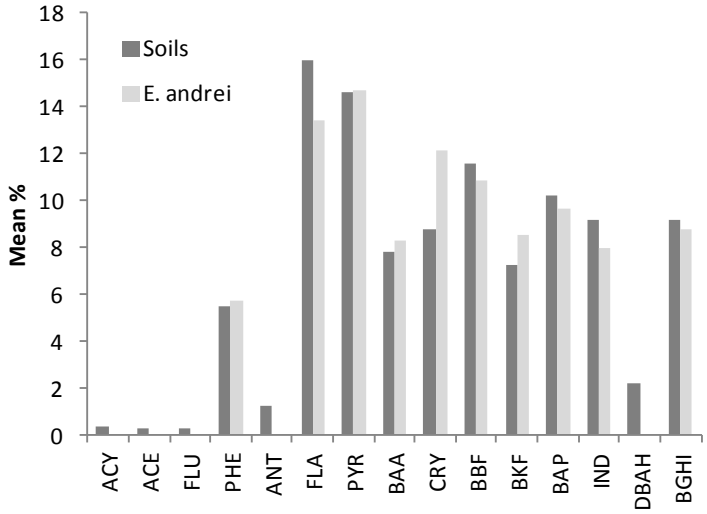

Figure 8.2 - Mean percentages of individual compounds in Lisbon urban soils (total extractions) and earthworm tissues.

The relationship between total concentration (∑10PAHs) in soils (normalized to OC) and concentration in earthworm tissues (lipid normalized) can be found in Figure 8.3. The relationship obtained between these two variables will correspond to the BSAF derived value for Lisbon samples; however the variability of results is high, especially for less contaminated soils.

Indeed, the BSAF value calculated for each sample varied as shown in Table 8.4, with the value for the ∑10PAHs ranging from 0.026 to 0.163 (kg_{OC}/kg_{lip}). This values are higher than the ones estimated by Parrish et al. (2006) for ∑4 PAHs in MGP soils: 0.011 (kg_{OC}/kg_{lip}) for *E. fetida* and 0.007 for *Lumbricus terrestris*. Other authors found BSAF values comparable with the ones of the present study. For example, Ma et al. (1998) report an average value of 0.1 (kg_{OC}/kg_{lip}) ranging from 0.03 to 0.26 for *L. rubellus* in 12 field contaminated soils. Higher values were obtained in field contaminated soils using the same species of the present study, being the average of 0.23 (kg_{OC}/kg_{lip}) (Jager et al., 2003). Nevertheless, these values are much lower than the one of the spiked experiment (1.65), reflecting the low bioavailability of PAHs in Lisbon soils.

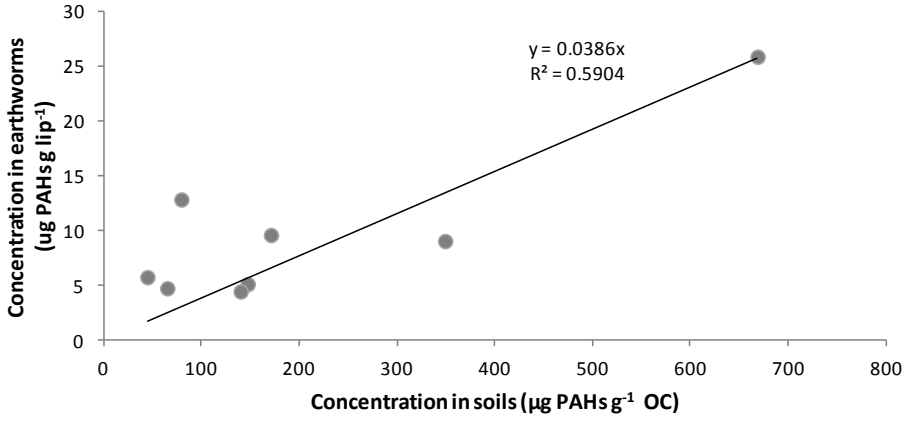

Figure 8.3 - Relationship between the ∑10PAHs in soils (OC normalized) and earthworms (lipid normalized).

The highest BSAF values were found in samples with the lowest concentrations of PAHs in soils (17, 18 and 57) and it appears to decrease as the concentrations in soils increase (Figure 8.4). It is interesting to note that this behaviour (higher availability in the lowest contaminated samples) was also observed for the water soluble content of soil samples. Since BSAF is a constant factor that is believed to be independent of the soil type, this higher values of availability in the lowest contaminated samples may suggest the presence of a more recent contamination as explained in the previous chapter, where the high contaminated samples were related with long-term accumulation. In fact, one of the main reasons for deviations of the EqPT is the sequestration of

pollutants in the soil (Sijm et al., 2000). Yet, higher accumulation percentages of PYR and PHE at lower contamination levels of soils with a similar aging period were reported by other authors (Chung and Alexander, 1999; Khan et al., 2011). One of the reasons for this behaviour could be that in low contaminated soils biological factors (e.g.: digestible organic matter) become of paramount importance (Barthe et al., 2008).

The BSAF is also expected to be independent of both the species and compound properties, such as the octanol-water partition coefficient (K_{OW}) (Krauss et al., 2000; Ma et al., 1998; Sijm et al., 2000). Indeed, in the present study it was observed that, rather than a single BSAF value for Lisbon soils, it seems that each sample has a BSAF value which is relatively constant for individual compounds (Table 8.4 and Figure 8.5). Krauss and Wilcke (2001) also observed that BSAF values were similar for all PAHs in urban soils, and Kreitinger et al. (2007) found similar values for the compounds with 4 to 6 rings (with exception of IND that was considerably lower).

Even so, it was observed some variability of the BSAF values within the same sample (Table 8.4 and Figure 8.5), and it seems that it is higher for the lowest contaminated samples (17, 18 and 57). In general, the highest BSAF values were observed for PHE, PYR, CRY and BKF. The differences observed between BBF and BKF could be due to coelution of the chromatographic peaks of these compounds during analysis, as referred in section 7.3.3. It is also interesting to note that CRY is one of the compounds with the highest BSAF values, as observed for the water soluble fraction.

Table 8.4 - BSAF values for the spiked soil and Lisbon urban soils.

Compound	Sample								
	Spike	4	12	17	18	22	23	49	57
PHE	0.65	0.028	0.026	0.224	0.154	0.027	0.040	0.057	0.085
FLA	2.40	0.026	0.016	0.176	0.137	0.024	0.037	0.044	0.059
PYR	2.38	0.041	0.021	0.212	0.152	0.028	0.043	0.049	0.066
BAA	2.25	0.036	0.033	0.180	0.111	0.035	0.040	0.062	0.078
CRY	2.52	0.060	0.046	0.228	0.141	0.044	0.045	0.083	0.089
BBF	1.37	0.031	0.034	0.119	0.130	0.036	0.029	0.058	0.069
BKF	1.94	0.052	0.033	0.203	0.121	0.031	0.043	0.066	0.103
BAP	1.56	0.030	0.026	0.137	0.117	0.033	0.038	0.056	0.072
IND	0.72	0.028	0.025	0.119	0.109	0.032	0.036	0.048	0.066
BGHI	0.41	0.031	0.026	0.118	0.112	0.042	0.044	0.051	0.074
∑PAHs	1.65	0.035	0.026	0.163	0.131	0.032	0.039	0.056	0.074
med	1.54	0.031	0.026	0.178	0.125	0.033	0.040	0.056	0.073
mean	1.46	0.036	0.029	0.172	0.128	0.033	0.039	0.057	0.076
min	0.41	0.026	0.016	0.118	0.109	0.024	0.029	0.044	0.059
max	2.52	0.060	0.046	0.228	0.154	0.044	0.045	0.083	0.103

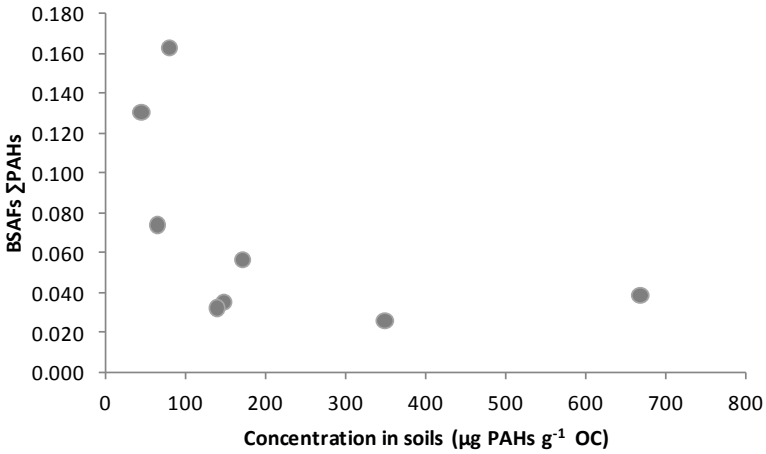

Figure 8.4 - Relationship between the BSAF values for the ∑PAHs and the levels of PAHs in soils (normalized to the OC content).

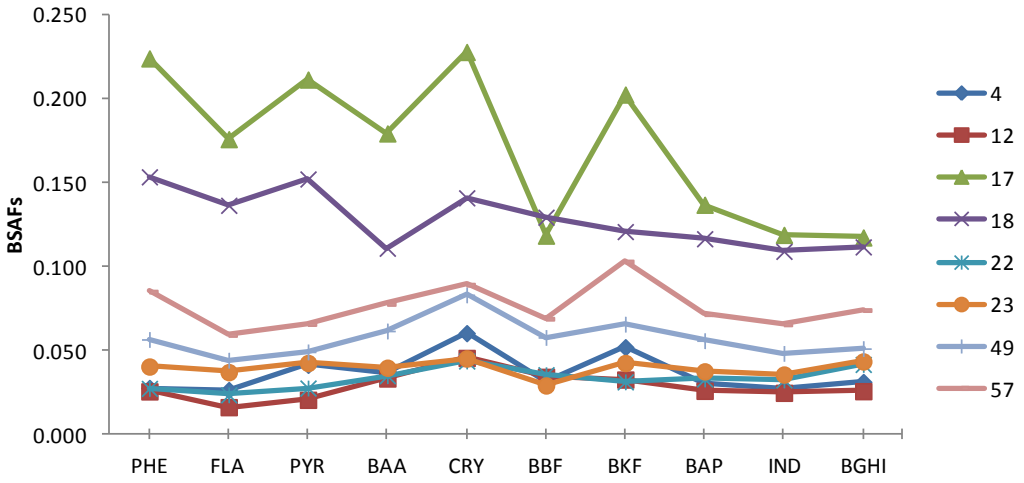

Figure 8.5 - BSAFs of individual PAHs compounds in earthworms for the 8 samples tested.

Although some studies reported an increase of the accumulated percentage of PAHs with the decrease of OC or soil OM (Sun and Li, 2005; Tang et al., 2002), no clear trend was observed in the present study. Yet, it seems that samples with the lowest content of OC show a higher variability of results than samples with highest OC contents (Figure 8.6), as observed also for the water soluble fraction (Figure 7.3). Other soil properties such as clay content have been suggested as important factors controlling the availability of PAHs in soils (Jager et al., 2003; Sun and Li, 2005). Indeed, Jager et al. (2003) observed that soils with a higher content of OC and clay, a low pH and

low PAHs level, showed higher BSAF values. However, in the present study it was not clear the influence of these soil properties, as previously reported by Hickman and Reid (2005).

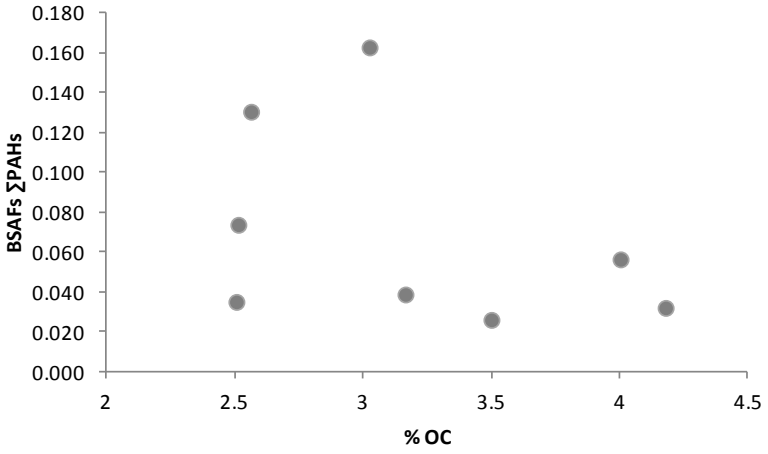

Figure 8.6 - Relationship between the BSAF values for the ∑PAHs and the OC content of soils.

8.3.4 Risk assessment implications

In the current risk assessment approach concentrations in earthworms (C_{org}) can be estimated from concentrations in soils (C_{soil}) based on EqPT as explained in Chapter 3, section 3.2.2. First, the pore water concentrations (C_{free}) are estimated using soil-water partition coefficients (K_{OC}) as shown in Eq. 8.2, and then the C_{org} is predicted using the bioconcentration factors (BCF) as shown in Eq. 8.3. BCF is generically assumed to be equal to K_{OW} (Gomez-Eyles et al., 2012; Jonker et al., 2007). The K_{OC} and K_{OW} values used in the following calculations were taken from Verbruggen (2012) and they are shown in Table 1 of Annex II.

Eq. 8.2

$$C_{free} = \frac{C_{soil}}{(f_{OC} \times K_{OC})}$$

Eq. 8.3

$$C_{org} = BCF \times C_{free}$$

The relationship between estimated levels using EqPT and measured concentrations for all compounds and in all samples tested can be found in Figure 8.7. In spite of the significant

relationship when considering all samples together, some differences between samples were observed (Figure 1 of Annex VII). For all samples except samples 4 and 17, the R^2 was greater than 0.5, and even higher than 0.8 for samples 18 and 23. As it can be observed in Figure 8.7, using the EqPT the concentrations in earthworms are overestimated (around 35 times) comparing with measured levels. This overestimation varied between samples and overall the overestimations were lower for samples with the lowest PAHs levels. In terms of different groups of compounds (3+4 ring and 5+6 ring) the relationship for each cluster as shown in Figure 2 of Annex VII remains very similar to the general relationship established with all samples as shown in Figure 8.7.

Considering the application of the previous approach to the case of the spiked sample (the aforementioned artificial soil), there is a slightly underestimation of the concentrations observed in organisms, but is very close to one (Figure 3 of Annex VII). For the 3 ring PAHs the prediction was also very close to 1 and a very good correlation was obtained (R^2=0.95), whereas for the 4 ring compounds, levels were underestimated by a factor of 5 but good correlations were observed (R^2=0.89). For the 5+6 ring compound there is no relationship between the observed and the estimated concentration (Figure 3 of Annex VII).

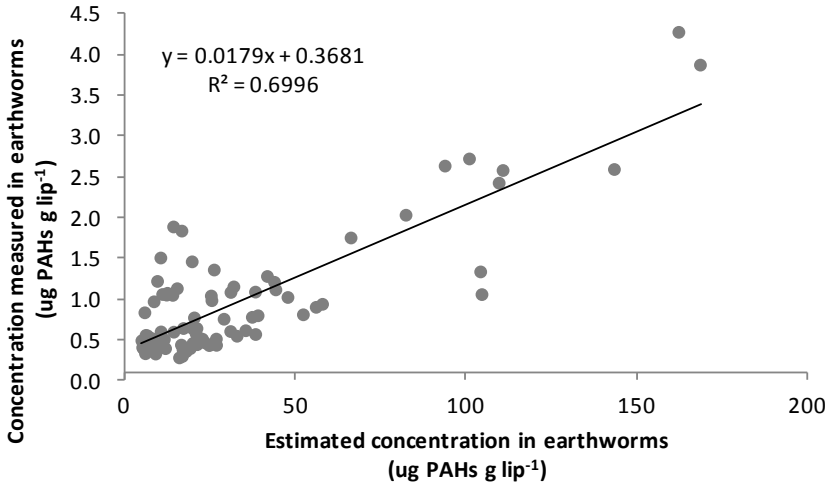

Figure 8.7 – Relationship between estimated PAHs concentration in earthworm using the EqPT and the measured concentrations.

The measured BSAF values in urban soils are much lower than predicted using EqPT, on the other hand, for spiked soils they were closer to the predicted value (Figure 8.8). Looking to Figure 8.8 it seems that there is a decrease in the BSAF for compounds with log K_{OW} higher than 5.5 for the measured value in both urban and spiked samples, whereas other authors observed the

opposite behaviour: an increase in the BSAF value with the increase in log K_{OW} (Jager et al., 2003). For compounds with log K_{OW} between 4.5 and 5.5 there is a slightly decrease of the measured BSAF value with the increase of K_{OW} for the urban soils, but for the spiked sample the opposite behaviour was observed. Comparing the BSAF values of spiked soil with the urban soils, the former were at least 8 times higher for PHE and 37 for FLA. These results highlight the low bioavailability of PAHs in urban soils comparing with spiked soils. Similar results were also observed by other authors (Kreitinger et al., 2007).

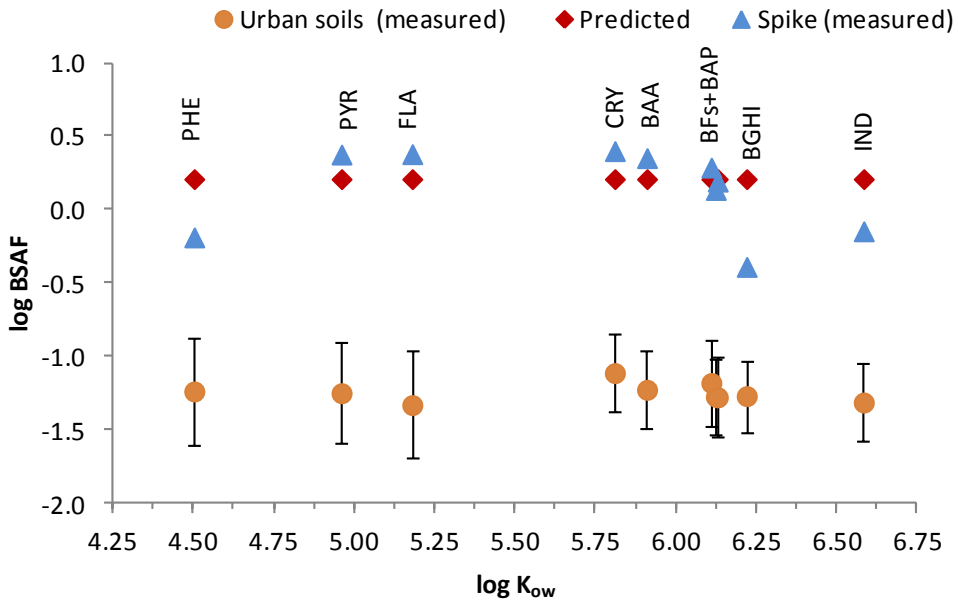

Figure 8.8 – Relationship between K_{OW} values and BSAFs of PAHs measured (mean and standard deviation for the 8 soils), estimated using EqPT and measured in a freshly spiked sample).

Considering a similar toxic mode of action (TMoA) for all PAHs, the sum of the internal concentrations of different compounds, normally expressed on a molar basis, gives rise to the same effect as that of a similar concentration of an individual compound, which is referred as concentration addition model (CAM). Therefore, individual concentrations in worm lipids were converted to a molar basis (mmol/kg lipid) and it was then possible to compare results with the literature threshold value known to cause toxicity due to narcosis (Critical Body Residue - CBR) (Jonker et al., 2007). In the present study the body residues measured were between 0.02 and 0.11 mmol/kg lipid for the 8 soil samples tested (Figure 8.9). These values are much lower than the literature levels reported as leading to lethality: 50 and 200 mmol/kg lipid (Verbruggen et al., 1999). Even considering the predicted concentrations, levels were between 0.3 and 4.7 mmol/kg

lipid. Considering the spiked sample, the measured value was 7 mmol/kg lipid. In the case of spiked sample, this measured level is slightly higher than the obtained using the predicted concentrations: 6.6 mmol/kg lipid. Considering the chronic toxicity limits HC_5 and HC_{50} suggested by Verbruggen (2012) it is possible to observe in Figure 8.9 that measured internal body residues of earthworms exposed to urban soils were always below both levels, whereas for predicted concentrations the values are lower than the HC_{50} but higher than HC_5 for 7 urban soils.

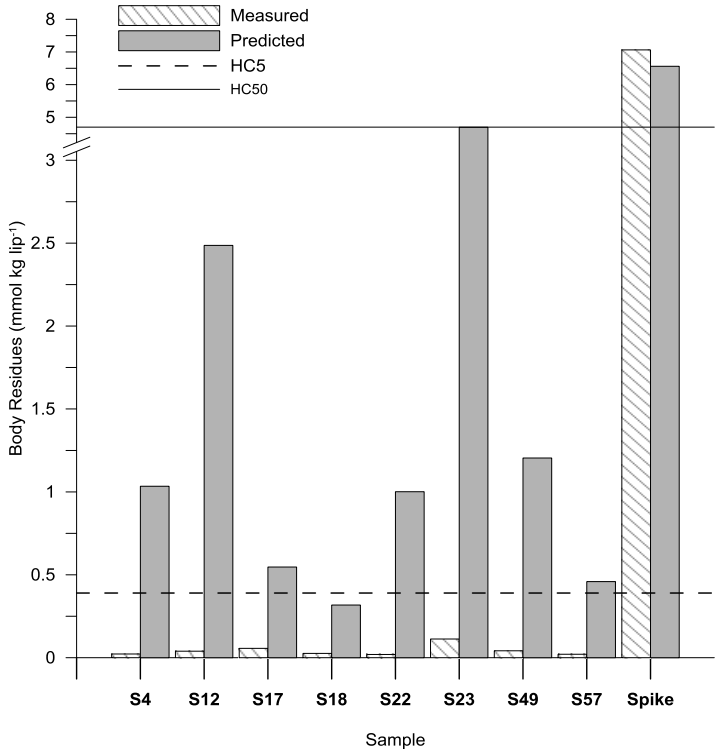

Figure 8.9 - Measured and predicted internal body residues of earthworms exposed to Lisbon soils and to a spiked sample. HC_5 and HC_{50} according to Verbruggen (2012) are also shown.

In spite of the overestimation given by the EqPT, this approach was applied to all samples to get an estimation of the potential risks associated with PAHs levels in earthworm body residues. Therefore, Eq. 8.2 and Eq. 8.3 were used to predict levels of PAHs in earthworm tissues in the 97 Lisbon urban soil samples. The boxplot of the body residues predicted levels, as well as the HC_5 and HC_{50}, are presented in Figure 8.10. Only for three samples the predicted level was above the HC_{50}, but lower than the lethal level referred previously. However, for several samples (24%) the predicted level is above the HC_5, and a further detailed risk assessment could be focused on these 23 samples.

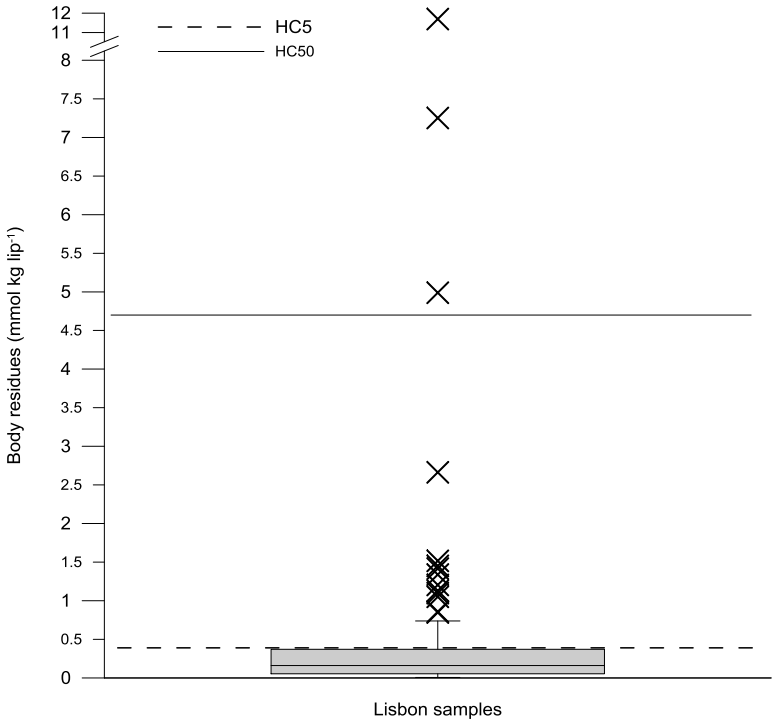

Figure 8.10 - Boxplot showing the predicted internal body residues for Lisbon soils. HC_5 and HC_{50} according to Verbruggen (2012) are also shown.

8.3.5 Comparison between chemical availability and bioavailability

The water soluble fraction of five of the samples tested for bioaccumulation was determined using the resin Tenax as explained in Chapter 7. In general, there is an increase of both bioavailable and water soluble content with the increase of total PAHs in soils samples (Figure 8.11). However, the relationship between these two methods is not very clear. In both cases only 10 PAHs were considered in the discussion, since the 3 ring compounds (with exception of PHE) and DBAH were not detected in the water extractions neither in earthworms.

When looking to concentration of individual compounds in each sample, only sample 23, which was the most contaminated from this set of samples, shows a clear relationship between the water soluble amount (OC normalized) and the concentration in tissues (lipid normalized) (R^2=0.74, data not shown). For all the other samples the coefficient of correlation was always below 0.5. Considering all samples together, a significant correlation was observed between the water soluble amount (OC normalized) and the concentration in tissues (lipid normalized) for the ∑10PAHs (R^2=0.58). On the other hand, when looking to individual compounds this is not always the case.

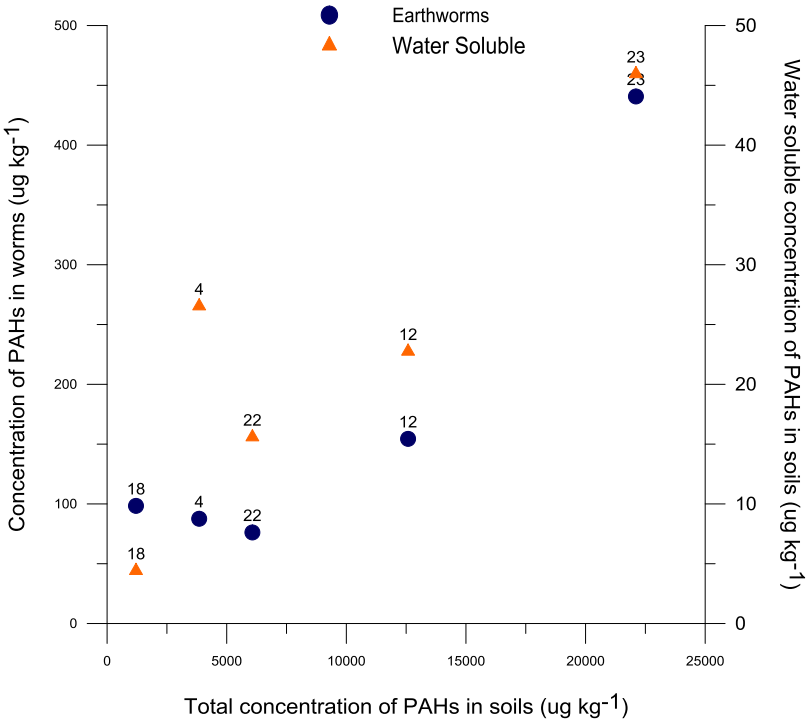

Figure 8.11 - Relationship between total PAHs concentrations and both earthworm and soil water soluble concentrations.

Figure 8.12 shows the relationship between water soluble and earthworm concentrations for two groups of PAHs: 4 and 5+6 ring compounds. For 4 ring compounds the relationship is very poor, being better for 5+6 ring compounds. Yet, in both cases, the concentration of compounds is much higher in earthworms than in the water soluble fraction. This underestimation of the water soluble content is greater for 5+6 ring compounds and this can be related to the importance of other routes of uptake rather than the soluble fraction, which become more important for compounds with higher K_{OW} (Ma et al., 1998).

In addition, the profiles of PAHs were different for earthworms, water soluble fraction and total extractions. Although the trend was similar, i.e., all profiles were dominated by 4 ring compounds followed by the 5+6 and 3 ring compounds, the earthworm profiles were much more similar to total extractions than to water soluble (Figure 8.13). In addition, total concentrations seem to correlate better with earthworm accumulation rather than water soluble content (Figure 4 of Annex VII).

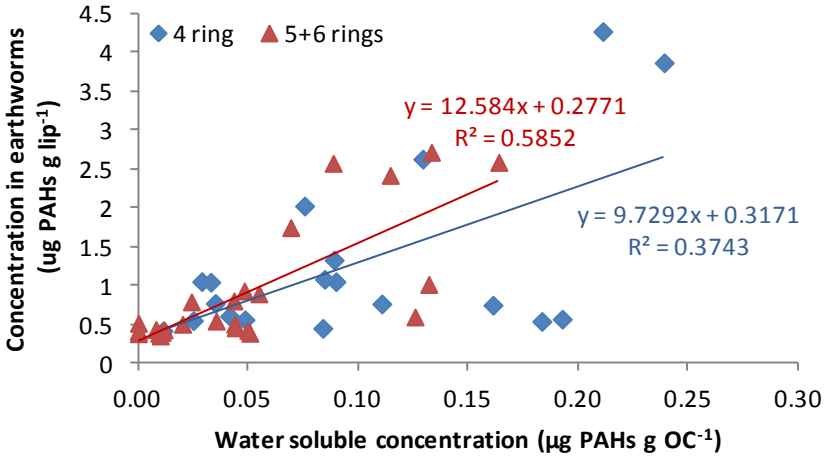

Figure 8.12 - Relationship between soil water soluble concentration (OC normalized) and earthworm concentrations (lipid normalized) for two groups of PAHs.

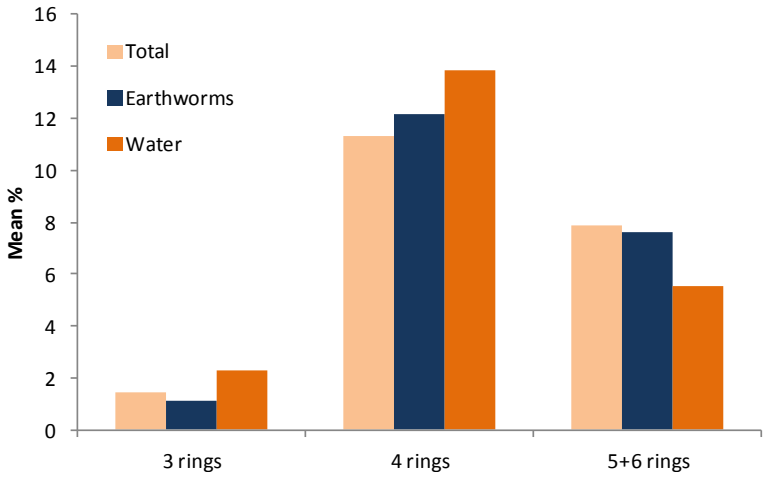

Figure 8.13 - Mean percentages of PAHs by ring number for soil total extractions, earthworm and soil water soluble fraction.

It was not observed a clear relationship between BSAF and the percentage of compounds desorbed in the water extractions. Moreover, BSAF values calculated using the soil water soluble fraction are much higher than observed with total concentrations (Figure 8.14). The BSFA values obtained in this way are divided by 2 because a 6h extraction is considered half of the rapidly desorbing fraction (Ten Hulscher et al., 2003). These values are, in general, higher than observed in the aforementioned spiked sample (section 8.3.2) and much higher than the estimated using the EqPT.

These results point towards the existence of much more complex mechanisms of earthworm accumulation than just uptake from soil solution, since earthworms could access compounds from both the aqueous and solid phase. As a matter of fact, it was observed that HMW compounds (especially the ones with 5 and 6 rings) are not likely to be leached from soils but they can be uptaked by organisms. Indeed, the existence of other routes of uptake rather than via passive diffusion from soil solution through outer membrane is another reason pointed for the deviations observed to the EqPT. For example Jonker et al. (2007) suggest that compounds can be transferred directly from soil solids to worm tissue by contact between the two phases, however this mechanism is not well studied. Other authors stated that pore water is the major route of exposure for compounds with log K_{OW}<5, but soil ingestion is very important for more hydrophobic compounds (Ma et al., 1998). Hence, these authors concluded that EqPT was applicable to predict the bioaccumulation of LMW PAHs in field contaminated soils, but not for the HMW.

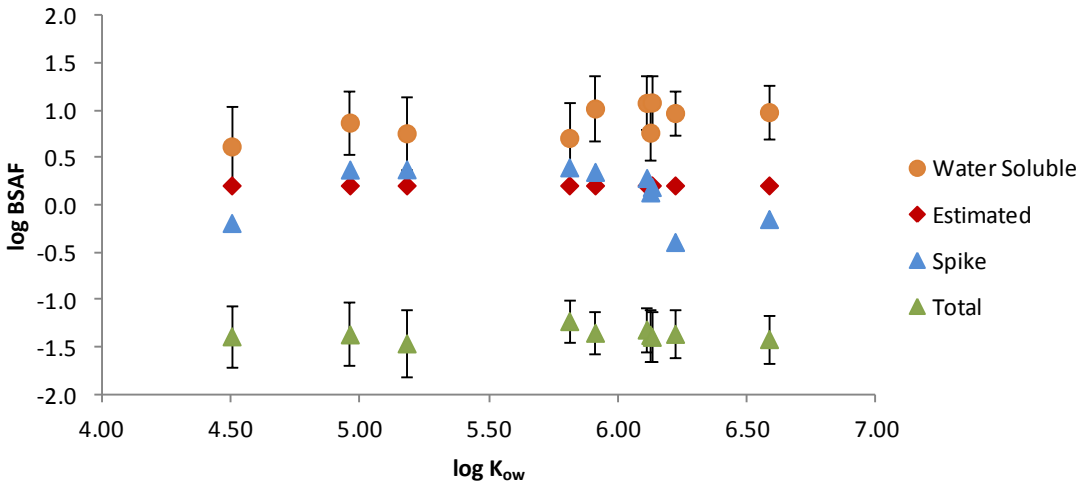

Figure 8.14 - Relationship between K_{OW} values and BSAFs of PAHs calculated using the soil water soluble fraction or total extractions and the measured concentration in earthworms (mean and standard deviation for the 5 soils), estimated using EqPT and measured in a freshly spiked sample).

Chapter 9

CONCLUSIONS AND FINAL REMARKS

The assessment of soil quality and characterization of potential risks to the environment and human health can be a very difficult task due to the heterogeneity and complexity of the matrix, the poor understanding about the fate of contaminants in the soil matrix, scarcity of toxicological/ecotoxicological data and lack of specific guidelines. In urban soils these difficulties are enhanced by the patchy nature of urban areas and the presence of complex mixtures of contaminants, which are mainly due to the long term accumulation of non toxic levels as a result of diffuse pollution caused by all the activities usually common in all the urban areas (e.g. traffic, industrial activity, and burning of carbon sources for heating). Yet, several tools are available which may help to assess the risks of soil contamination in a simpler, cost effective and reliable way. However, there is still much to improve and a long way to go in the field of risk assessment of hydrophobic organic contaminants (HOCs) in soils.

The most important and useful tools are the risk assessment (RA) frameworks. The tiered approach of the Dutch framework in particular, which generates data in sequential steps with increasing complexity, can be a very cost-effective tool for managing contaminated sites. The RA allows identifying the contaminants of potential concern and receptors at risk, and by combining expected exposure levels and expected effects it becomes possible to obtain a risk characterization for a given area/environmental matrix.

The initial problem formulation, in which the contaminants of potential concern are targeted, is a particularly hard task when dealing with urban areas. Two major groups of contaminants are normally found at potentially hazard levels in urban soils (potentially toxic elements - PTEs and HOCs). Usually, PTEs of concern include Cu, Hg, Pb, and Zn while the organic contaminants include PAHs, PCBs and pesticides. The present study focused only in two groups of HOCs (PAHs and PCBs), which due to their sources, are known to be present in urban areas. Besides, their environmental significance was also crucial for the selection step.

At the step of initial problem definition it is also important to identify the origin of contamination, which in this case is expected to be anthropogenic (e.g. traffic and industry) or natural (e.g. telluric and natural fires), pathways, receptors, and routes of exposure. The main pathway of these organic contaminants into soils is atmospheric deposition, but dusts (from pavements, tires and brakes), leakages from automobiles and direct disposal may also become important. Once in soils, contaminants may be recirculated by volatilization, leached through the soil solution, and uptake by biota. Dusts, which can be formed either by soil derived material or by deposition of particles, can be transported outside urban areas or to nearby aquatic systems. For both soils and dusts, runoff can play an important role in the transport of HOCs within and to

outside urban areas. The receptors are the aquatic bodies, soil organisms, vertebrates and humans. The pathways are the inhalation, ingestion and dermal contact of soil and dusts. In urban areas there is also a large variety of land uses, with different patterns of source-pathway-receptor, such as roadsides, residential areas, gardens, and parks.

The first step to assess the potential exposure of contaminants is to measure their total levels in selected matrices. In addition, the identification of contaminant sources is also a critical step in HOCs risk assessment and management. Multivariate statistics or simple profiles of compounds and isomer ratios are normally used for source identification, although they are not free of some serious drawbacks (Cachada et al., 2009; Ravindra et al., 2008; Yunker et al., 2002). Specifically for PAHs these approaches have been used to provide information on the possible origin (petrogenic *versus* pyrogenic) and sources of contamination (e.g.: tire debris, biomass, and fuel combustion). The relationship between HOCs contamination and PTEs can also provide indications on their sources. Some studies reported that HOCs and PTEs contamination can be related (Cachada et al., 2012b; Maliszewska-Kordybach et al., 2009), but most studies on urban soil quality do not take into account both types of contamination (Jiang et al., 2011; Peng et al., 2011a; Wu et al., 2011). Soil properties are also often excluded from urban soil studies and as a consequence their role on HOCs fate in soils is not fully understood.

The present work studied two contrasting urban areas, Lisbon and Viseu, which differ in geological and climatic conditions, industrial and urban development processes. A comprehensive study on the above mentioned contaminants was conducted in the two urban areas in order to: a) assess their levels and their potential risks to human health and to the environment; b) identify their sources and respective relevance; c) study their behaviour particularly in urban soils. Furthermore, a deeper study about the behaviour of PAHs in Lisbon urban soils was performed, which included the study of levels of PAHs in street dusts.

The concentrations of PAHs and PCBs were related to the size of the city. In general, concentrations of HOCs in Lisbon were high (mean value of 1,544 μg kg^{-1} for PAHs and 7.0 μg kg^{-1} for PCBs), whereas in Viseu they were low (mean value of 169 μg kg^{-1} for PAHs and 4.6 μg kg^{-1} for PCBs), when compared with other urban areas worldwide. The high variability of the results observed might be a consequence not only of the proximity to sources, source type, age of sites (time for cumulative deposition) but also of the retention capacity of soils. The reason for the occurrence of lower concentrations of HOCs in Viseu is likely to be the size of the town and the lack of a major industrial activity. Nevertheless, levels of PCBs in this town were not as low as expected and atmospheric transport should be the main reason. This hypothesis is supported by

the differences observed in the HOCs profile of the two cites. Even so, differences in climate should also be taken into consideration, since it can affects the volatilization of lower molecular weight compounds. Traffic, domestic heating, agriculture practices, and forest fires may also be a source of PAHs in Viseu urban soils. On the other hand, in Lisbon local high temperature sources of HOCs, such as industry and incineration, and traffic, are of utmost importance as observed also by their relationship with PTEs. Cluster analysis revealed that PAHs and PCBs were associated with anthropogenic toxic elements (Cu, Pb, Zn, and Hg in Lisbon; Pb and Hg in Viseu), therefore reflecting common sources. The influence of organic carbon (OC) on the retention of HOCs is not very clear yet; nevertheless, it was observed in present study that OC has an effect on the fate of PAHs in both cities and also affects the high chlorinated PCB in Lisbon soils. Some limitations, particularly the presence of numerous contaminant sources and multiple weathering processes (which affect contaminants profiles and their relationships), led to difficulties on source identification and consequently some of the sources were not clearly defined in some cases.

As in the first sampling campaign, the distribution of PAHs in Lisbon soils from the second sampling campaign was characterized by a low median value (714 μg kg^{-1}) and by the presence of several hotspots, being the maximum value as high as 73,395 μg kg^{-1}. The results of the two sampling campaigns differed mostly by the presence of hotspots with higher concentrations in the second campaign. The main reason was that this last campaign included samples from the airport and it was more focused in city centre where the concentrations were in general higher than in any other places. However the pattern of the spatial distribution remains very similar. In addition, the PAHs profiles and isomer ratios remain unaltered meaning that the first sampling gave a good picture of the state of Lisbon soils contamination.

The study of levels of PAHs in street dusts allowed obtaining a clear identification of the sources of PAHs in urban areas. For that, the spatial distribution and origin of PAHs were also further investigated. Levels of PAHs in street dust showed the trend of soils, with a low median value (671 μg kg^{-1}) but a wide range of values (71 - 84,321 μg kg^{-1}). Nevertheless, when looking at each site, concentrations in soils and street dusts were not always related. Concentrations in soils were in general higher than in dusts, and this may indicate that soils are the ultimate sink of HOCs in urban areas.

Most of studies performed on street dusts had the aim of identifying specific sources of PAHs and therefore samples were normally collected nearby probable sources. Since in this study the aim was to assess the risk of street dusts to the environment and human health, the sampling campaign was designed in order to obtain a coverage as broad as possible of the entire urban

area. As a result, source identification became again a very difficult task. The comparison of PAHs profiles of soils and dusts gave interesting results. Soils were enriched in compounds with 5 and 6 rings (48±9%) comparing with street dusts (41±7%), supporting the hypothesis of the long term accumulation of PAHs in urban soils, since these compounds are difficult to be evaporated and degraded and therefore they become enriched in soils. On the other hand, low molecular weight (LMW) PAHs showed a higher percentage in street dusts (15±5%) over soil samples (10±7%), suggesting a more recent contamination of this matrix since they are easier to be evaporated and degraded. Yet, this difference can also be related to specific sources of PAHs in dust samples. In addition to profiles, isomer ratios also differ for the two matrixes, reflecting the different origin of dusts (soil-derived and anthropogenic material, as well as particles released into the atmosphere due to natural sources and anthropogenic activities). Other sources that could be identified were traffic, tire, and asphalt debris. When including PTEs in the multivariate statistics, differences between the two matrixes remain and the complexity of dust samples due to the presence of several sources were highlighted.

Other interesting tools that can be used to integrate information of large datasets are the geostatistical methods. These methods can be very useful for site characterization, for example to evaluate the extent of contamination or to identify sources of contamination. Since urban soils can be very heterogeneous and because it is not always possible to sample at the desirable location, it is very difficult to obtain a "real picture" of the area. In order to overcome this difficulty, unknown values can be estimated from data taken at specific locations being the most useful spatial analytical techniques the kriging interpolation. However, in order to apply these tools a high number of samples are needed. Considering this, results of the two sampling campaigns were used with the purpose of better understanding the spatial distribution and major inputs of those contaminants. The prediction map obtained allowed a clear identification of areas with the highest levels of PAHs. It was also possible to build the maps of the factor scores obtained from the factor analysis, which show the areas most affected by telluric and anthropogenic inputs. It was clearly shown that the areas with the highest PAHs concentrations were the ones close to the airport and the river (city centre). The latter area is the oldest part of the city and has a high population density. The high concentrations found in soils from the city centre should be a result of long term accumulation due to diffuse pollution mostly from traffic (through atmospheric emissions, tire debris and fuel exhaust, as well as pavement debris). Indeed most of the sites sampled in the city centre were historical gardens and parks. Specific sources of

PAHs were also identified: railway, crematoriums, hospital waste incinerator, the docks, and the shipyard activities.

As a first tier of RA, simple comparisons with soil quality criteria (generic guidelines, guidelines for protection of environment or human health) may be enough to identify the major problems. The output of these comparisons is normally called hazard quotient (HQ) and in the case of environmental RA (ERA), it is also suggested to calculate the toxic units (TU) (comparison with PNEC values). Despite being normally very conservative approaches, since it generally overestimates risks, it is a simple way to identify substances that can be a threat and areas in need of a more detailed RA. Still, this approach is reliable if guidelines are really conservative, otherwise risks can be underestimated. This methodology was followed in the first study that was conducted in the two urban areas (Lisbon and Viseu) and considering several guideline values, this assessment allowed to conclude that unacceptable effects on organisms are likely to occur as a result of the exposure to PAHs present in soils from some Lisbon areas.

In the present study, the evaluation of the potential risks that HOCs pose to human health was also made, by calculating the non-cancer risks as well as cancer incidence and the mutagenic risks resulting from the exposure to urban soils during daily and recreational activities. Despite the several limitations (e.g. methodology may not reflect the Portuguese reality; low number of samples; high associated uncertainty) of this approach, it is an indication of the potential hazard. The incremental probability of developing cancer over lifetime, based on a reasonable maximum exposure to PAHs present in Lisbon soils, for a residential or occupational land use was slightly higher than the target risk of one in one million inhabitants (10^{-6}). Similarly, the mutagenic risk of PAHs in Lisbon for residential and recreational areas was also slightly higher than the target risk. Viseu, in spite of the low contamination levels, showed mutagenic risks due to PAHs very close to the target value, but for all other scenarios the risks were negligible. PCBs risks were below the levels of concern, with similar values in the two cities. These results are in line with the ones found when comparing with the guidelines and to the calculation of HQ and TU: only some Lisbon samples may represent a potential hazard regarding their PAHs levels and a further study should be performed. Whether the Lisbon soils are strongly affected or not by PAHs contamination will be dependent of the soil screening guidelines used.

When applying this simple approach of comparing different guideline values and PNECs with data gathered from the two sampling campaigns, it was interesting to note that results remain very similar. In spite of higher values for TU and HQ obtained, due to the presence of hotspots with very high levels in the second campaign, the percentages of samples that exceed the unit

remain very similar. Again, this means that the first sampling produced a good picture of the state of Lisbon soils contamination. However, this high number of samples allowed building contour maps of PAHs levels in soils with the prediction of the areas that will exceed guidelines. In addition, regarding ERA, it was possible to obtain contour maps with distribution of the multisubstance potentially affected fraction (mSPAF) of species. This mSPAF value refers to the cumulative risks of mixtures and allows quantifying the fraction of species that is expected to be locally exposed beyond the selected effect level and in which the ecological function of soils are likely being threatened. Regarding human health RA, it was possible to obtain contour maps with the prediction of areas that exceed the levels of BAP of several guidelines, as well as with the predicted areas that exceed the total lifetime cancer risk (TLCR). These maps can be used, for example, to cross information of sensitive land uses and identify most problematic areas (e.g. playgrounds and schools). Therefore, these spatial analysis tools can be very useful to relate risks to the areas potentially affected, which may be applied in risk management, to help risk managers and local authorities when planning the urban area. In addition, these approaches can be useful to integrate spatial information on contaminant concentrations and land use.

The potential risks to the environment of street dusts contamination were also assessed, by the same methods referred previously. This approach is very questionable once there are no guidelines for dusts. Therefore, only few guidelines were selected according to the most relevant routes of exposure: food and soil ingestion by wildlife and for freshwater protection. The major risks are likely to be if the dusts reach the aquatic environment. It is known that street dusts may be an important diffuse source through runoff into aquatic environment resulting in an enrichment of contaminant levels in sediment, being this phenomenon especially important in riverine or coastal areas such as Lisbon. Concluding, the impact of street dust on the environment should be further assessed, especially in the case of the nearby estuary.

The risks of street dusts to human health were also calculated since street dusts represent a more realistic exposure route than soils. Indeed, in addition to be in more direct contact with population than soils, they also tend to be more easily transferred through ingestion, inhalation, and dermal contact due to their low particle size. Results show that the TLCR is higher than 10^{-6} for a residential and occupational exposure, when considering the 95% percentile of the upper confidence on the mean. Although that these values were strongly influenced by the airport samples, the TLCR remained above the recommended after excluding these hotspots. The models used to calculate the TLCR are very sensitive to the default values used and in the present study risks were mostly calculated using the values from USEPA and it should be verified whether the

exposure parameters are adequate to the Portuguese reality. These models could also be improved by determining the oral and inhalation bioaccessibility of samples that exceed the target risk.

As it was possible to verify, there are several problems regarding the RA approach, such as uncertainties and validation of models. Another major issue is the availability of contaminants, which may cause significant deviations in the methodologies described previously. Therefore, the evaluation of the available fraction of HOCs is of utmost importance for assessing their risk to the environment and human health. This available fraction, which can be solubilised and/or easily extracted, is believed to be the most accessible for bioaccumulation, biosorption and/or transformation by organisms. Based on this, two main types of chemical methods have been developed, closely related to the concepts of bioaccessibility and freely available concentrations: non-exhaustive extractions and biomimetic methods. From the review of the processes involved on PAHs availability to microorganisms, earthworms and plants and the outputs given by the different chemical methods it became clear that the suitability of chemical methods to predict bioavailability of the 16 priority PAHs in dissimilar field contaminated soils was not yet demonstrated, being especially difficult for high molecular weight compounds. Even though the potential to predict microbial mineralization using non-exhaustive extractions is promising, it will be very difficult to accomplish for earthworms and plants, due to the complexity of accumulation mechanisms that are not taken into account by chemical methods. Furthemore, regarding bioaccessibility, the experimental details need to be clarified and standardized. After obtaining consistent results for some methods, then they can be chosen on a basis of their reproducibility, speed, practicability, and cost.

Since some hotspots of PAHs contamination have been identified in Lisbon urban area, they were further investigated for the water soluble fraction of these contaminants. Therefore, a solid phase extraction method with Tenax-TA adsorbent was applied to assess the water soluble fraction of PAHs in selected soil samples. Although it is believed that this water soluble fraction is the most important when estimating potential risks to environment and human health, there is not enough data to associate this fraction with the bioavailable fraction. Therefore, the Tenax extractions seem more relevant to evaluate the mobility of PAHs in soils than to predict the bioavailable fraction. In spite of the very high total PAHs concentrations, the water soluble fraction was very low. The samples used to assess the water soluble fraction were some of the most contaminated ones and they were mostly taken from old parks and gardens from the city centre. Therefore, the low mobility of PAHs in selected samples support the hypothesis of a long-

term accumulation, since the low available fraction suggests that PAHs experienced sequestration after prolonged residence times in soil, called aging. The relationship between the water soluble fraction and the soil properties is not very clear, and the low number of samples as well as other factors such as the different aging of soil contaminants and the nature of organic matter may contribute to these findings. The profiles and percentages of available compounds seem to reflect the compounds physico-chemical properties: compounds with 3 and 4 rings are more likely to be transferred to soil solution than the ones with 5 and 6 rings.

The approach for the inclusion of chemical availability data in risk assessment, by directly comparing the concentrations obtained in the non-exhaustive extractions with soil screening levels indicates that PAHs in Lisbon urban soils are not a potential hazard. However, this approach is based on the fact that soil screening levels are obtained using freshly spiked soils and therefore no aging is likely to occur, and to use this direct comparison it is necessary that the non-exhaustive method extracts around 100% of the freshly spiked concentrations. However, this is probably valid for some compounds but for very hydrophobic contaminants this may not be true. Therefore, the extraction efficiency of the individual PAHs from freshly spiked soils using a water based Tenax extraction should be tested in advance.

Bearing in mind that bioavailability is compound and species specific, to find a chemical method to predict bioavailability of the mixture of compounds usually present in urban soils will be difficult. Thus bioaccumulation assays were used in order to investigate the bioavailability of PAHs to earthworms. Results showed that, as for the water soluble extractions, the availability of PAHs to *E. andrei* was very low. In spite of no significant relationship found between soil properties and bioavailability, it was verified that the biota-to-soil accumulation factors (BSAF) were sample dependent rather than compound dependent, and it was higher for low contaminated samples. These finding reinforce the assumption that the high levels of PAHs in Lisbon urban soils are a result of long term accumulation resulting in an aging of the compounds and therefore in their low bioavailability. When comparing BSAF values obtained experimentally with the ones calculated using the equilibrium partition theory (EqPT), it was verified that the estimation will result in an overestimation of risks. Considering this, the traditional approach of estimating levels using EqPT could be seen as a conservative approach of calculating risks. However, when comparing the estimated results with freshly spiked soils a slightly underestimation was observed. Therefore, if the contamination is recent the real risk could be higher than predicted.

It would be expected that non-exhaustive extractions will give a better prediction of available concentrations than total extractions. However, when comparing the water soluble concentrations in soils with the earthworm's accumulation the relationship was poor, being better when total concentrations are considered. Moreover, it was observed that the soil water soluble fraction underestimates the bioavailable fraction. In addition, the PAHs profiles found in earthworms are much more similar to total extractions than to water soluble. These results point towards the existence of much more complex mechanisms of earthworm accumulation than just uptake from soil solution, since earthworms could access compounds from both the aqueous and solid phase.

The use of biomimetic methods could be useful and reliable for example to improve the EqPT by calculating site-specific soil organic carbon-water partition coefficient (K_{OC}) values. Even so, the determination of species-specific lipids-water partition coefficients should also be considered. Another important problem is biotransformation or metabolism of PAHs and how they affect the bioaccumulation measurements. Future work will also require accurate measurements of kinetics of uptake and elimination data for key species, compound and species-specific bioconcentration factors.

The use of laboratory spiked soils may be an initial approach to understand contaminants behaviour but they are not the most appropriate regarding how to understand their behaviour in field contaminated samples. In spite of most of the chemical methods are able to respond to aging effects, soil properties and compounds behaviour, no consistent data was obtained in some cases. Due to the low number of studies that dealt with a heterogeneous number of field PAHs contaminated soils, there is a need to test chemical methods in such conditions. In addition, due to the different behaviours of individual PAHs, there is also a need to perform studies (both in spiked and field contaminated soils) with the 16 individual PAHs or at least with a representative compound of each group of PAHs. Finally, since several factors may play an important role in controlling availability, including the quality of the natural organic matter, the predictability of chemical methods should be tested in several dissimilar soils and not only in few soil types as most studies previously performed.

In conclusion, performing a chemical screening as a first tier could be a simple and effective approach to start an RA. The simple comparison with guideline values can be an easy way to identify substances of concern. It is expected that this approach leads to protective values since most of guidelines were derived based on ecotoxicological assays performed in freshly spiked soils and considering the worst case scenario for human health protection. Indeed, this link between

laboratory assays and field conditions is crucial. In addition, reliable guidelines are needed and the harmonization of RA approaches among different countries would be an important asset. Moreover, this is only applicable if all contaminants of potential concern are identified and screened, and in the case of diffuse pollution such as urban soils the effect of mixtures is of upmost importance. Further steps should be conducted in the areas where there is a potential risk. This site-specific risk assessment should include the chemical availability of contaminants (although this is far from being clarified and standardized) and relevant ecotoxicological tests.

Chapter 10
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Annex I

Table 1 – Characteristics of the 16 priority PAHs studied in this work.

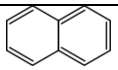
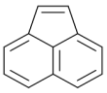
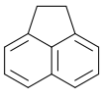
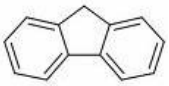
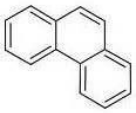
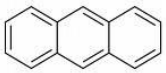
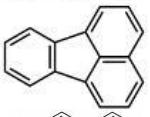
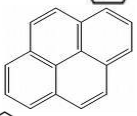
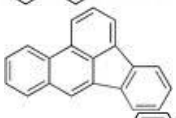
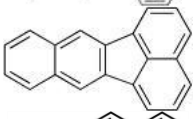
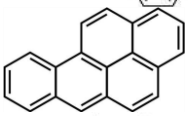
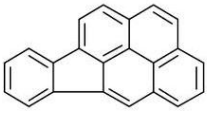
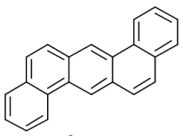
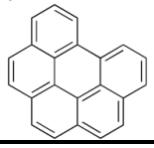
Name	Abbreviation	Empirical formulae	Structure	Nº rings	Molecular ion (m/z)
Naphthalene	NP	$C_{10}H_8$		2	128
Acenaphthylene	ACY	$C_{12}H_8$		3	152
Acenaphthene	ACE	$C_{12}H_{10}$		3	154
Fluorene	FLU	$C_{13}H_{10}$		3	166
Phenanthrene	PHE	$C_{14}H_{10}$		3	178
Anthracene	ANT	$C_{14}H_{10}$		3	178
Fluoranthene	FLA	$C_{16}H_{10}$		4	202
Pyrene	PYR	$C_{16}H_{10}$		4	202
Benzo(a)anthracene	BAA	$C_{18}H_{12}$		4	228
Chrysene	CRY	$C_{18}H_{12}$		4	228
Benzo(b)fluoranthene	BBF	$C_{20}H_{12}$		5	252
Benzo(k)fluoranthene	BKF	$C_{20}H_{12}$		5	252
Bezo(a)pyrene	BAP	$C_{20}H_{12}$		5	252
Indeno(1,2,3-cd)pyrene	IND	$C_{22}H_{12}$		6	276
Dibenzo(a,h)anthracene	DBAH	$C_{22}H_{14}$		5	278
Benzo(ghi)perylene	BGHI	$C_{22}H_{12}$		6	276

Table 2 - Characteristics of the 21 PCBs studied in this work (indicator congeners in bold).

Name	IUPAC #	Empirical formulae	Molecular ion (m/z)
2-Chlorobiphenyl	1	$C_{12}H_9Cl$	188
2,3-Dichlorobiphenyl	5	$C_{12}H_8Cl_2$	222
2,2',5-Trichlorobiphenyl	18	$C_{12}H_7Cl_3$	256
2,4',5-Trichlorobiphenyl	31	$C_{12}H_7Cl_3$	256
2,4,4'-Trichlorobiphenyl	**28**	$C_{12}H_7Cl_3$	256
2,2',5,5'-Tetrachlorobiphenyl	**52**	$C_{12}H_6Cl_4$	292
2,2',3,5'-Tetrachlorobiphenyl	44	$C_{12}H_6Cl_4$	292
2,3',4,4'-Tetrachlorobiphenyl	66	$C_{12}H_6Cl_4$	292
2,2',4,5,5'-Pentachlorobiphenyl	101	$C_{12}H_5Cl_5$	326
2,2',3,4,5'-Pentachlorobiphenyl	87	$C_{12}H_5Cl_5$	326
2,3,3',4',6-Pentachlorobiphenyl	**110**	$C_{12}H_5Cl_5$	326
2,3',4,4',5'-Pentachlorobiphenyl	**118**	$C_{12}H_5Cl_5$	326
2,2',3,5,5',6-Hexachlorobiphenyl	151	$C_{12}H_4Cl_6$	360
2,2',4,4',5,5'-Hexachlorobiphenyl	**153**	$C_{12}H_4Cl_6$	360
2,2',3,4,5,5'-Hexachlorobiphenyl	141	$C_{12}H_4Cl_6$	360
2,2',3,4,4',5'-Hexachlorobiphenyl	**138**	$C_{12}H_4Cl_6$	360
2,2',3,4',5,5',6-Heptachlorobiphenyl	187	$C_{12}H_3Cl_7$	394
2,2',3,4,4',5',6-Heptachlorobiphenyl	183	$C_{12}H_3Cl_7$	394
2,2',3,4,4',5,5'-Heptachlorobiphenyl	**180**	$C_{12}H_3Cl_7$	394
2,2',3,3',4,4',5-Heptachlorobiphenyl	170	$C_{12}H_3Cl_7$	394
2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl	206	$C_{12}H_1Cl_9$	464

Table 3 - Genotoxicity and carcinogenicity of PAHs and TEF values.

Compound	Genotoxicity[a]	USEPA[b]	IARC[c]	RIVM[d]	TEF[e]
NP	No	C		R40/R22	0.001
ACY	Q[a]	D		-	0.001
ACE	Q[a]	-		-	0.001
FLU	No	D	3	-	0.001
PHE	Q[a]	D	3	-	0.001
ANT	No	D	3	-	0.01
FLA	Yes	D	3	-	0.001
PYR	Q[a]	D	3	-	0.001
BAA	Yes	B2	2A	R45	0.1
CRY	Yes	B2	3	R45/R68	0.01
BBF	Q[a]	B2	2B	R45	0.1
BKF	Yes	B2	2B	R45	0.1
BAP	Yes	B2	2A	R45/R46/R60/R61	1
IND	Yes	B2	2B	-	0.1
DBAH	Yes	B2	2A	R45	1
BGHI	Yes	D	3	-	0.01

[a] Q=questionable, results derived from a small database (WHO, 2003);

[b] B2, probable human carcinogen - based on sufficient evidence of carcinogenicity in animals; C, possible human carcinogen; D, not classifiable as to human carcinogenicity (IRIS, 2014)

[c] 2A, possible human carcinogens; 2B, probable human carcinogens; 3, no data on carcinogenesis in humans and limited or inadequate data in animals (WHO, 2003);

[d] R40, limited evidence of carcinogenic effect; R22, harmful, toxic or very toxic if swallowed; R45, may cause cancer; R46, may cause heritable genetic damage; R60, may impair fertility; R61, may cause harm to unborn child; R68, possible risk of irreversible effects (Verbruggen, 2012)

[e] Tsai et al., 2004

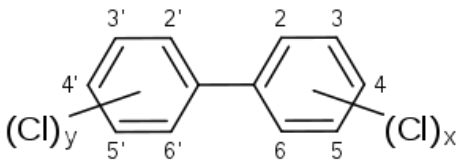


Figure 1 – General structure of PCBs.

Risk Assessment Equations

1. Total lifetime carcinogenic risk

1.1 Residential soils

1.1.1 Mutagenic risk

a) Ingestion

$$LR_{ing} = \frac{C_{soil} \times EF \times IFSM_{ad}}{AT \times 10^6} \times CSF_o$$

Where:

$$IFSM_{ad} = \frac{ED_{0-2} \times IRS_c \times 10}{BW_C} + \frac{ED_{2-6} \times IRS_c \times 3}{BW_c} + \frac{ED_{6-16} \times IRS_a \times 3}{BW_a} + \frac{ED_{16-30} \times IRS_a \times 1}{BW_a} (mg\ y\ kg^{-1}d^{-1})$$

b) Dermal contact

$$LR_{der} = \frac{C_{soil} \times EF \times DSFM_{ad} \times ABS}{AT \times 10^6} \times \frac{CSF_o}{GIABS}$$

Where:

$$DSFM_{ad} = \frac{ED_{0-2} \times SA_c \times AF_c \times 10}{BW_C} + \frac{ED_{2-6} \times SA_c \times AF_c \times 3}{BW_C} + \frac{ED_{6-16} \times SA_a \times AF_a \times 3}{BW_a} + \frac{ED_{16-30} \times SA_a \times AF_a}{BW_a} (mg\ y\ kg^{-1}d^{-1})$$

c) Inhalation

$$LR_{inh} = \frac{C_{soil} \times EF \times ET}{AT \times PEF} \times [(ED_{0-2} \times IUR \times 10) + (ED_{2-6} \times IUR \times 3) + (ED_{6-16} \times IUR \times 3) + (ED_{16-30} \times IUR \times 1)]$$

Where:

- LR_{ing} = Total risk through ingestion
- LR_{der} = Total risk through dermal contact
- LR_{inh} = Total risk through inhalation
- GIABS = 1
- 10^{-6} correction factor (mg kg^{-1})
- PEF = 1.36×10^9 (m^3 kg^{-1})
- Other values can be found in Table S1 and Table S2

d) Total lifetime mutagenic risk (TLMR)

$$TLMR = TR_{ing} + TR_{der} + TR_{inh}$$

1.2 Outdoor worker soil – cancer risk

a) Ingestion

$$LR_{ing} = \frac{C_{soil} \times EF \times ED \times IRS}{AT \times Bw \times 10^6} \times CSF_o$$

b) Dermal contact

$$LR_{der} = \frac{C_{soil} \times EF \times ED \times SA \times AF \times ABS}{AT \times Bw \times 10^6} \times \frac{CSF_o}{GIABS}$$

c) Inhalation

$$LR_{inh} = \frac{C_{soil} \times EF \times ED \times ET}{AT \times PEF} \times IUR$$

1.3 Recreational soil

1.3.1 Cancer risk

a) Ingestion

$$LR_{ing} = \frac{C_{soil} \times IFS_{ad}}{AT \times 10^6} \times CSF_o$$

Where:

$$IFS_{ad} = \frac{ED_c \times EF_c \times IRS_c}{BW_C} + \frac{ED_a \times EF_a \times IRS_a}{BW_a} (mg\ kg^{-1})$$

b) Dermal contact

$$LR_{der} = \frac{C_{soil} \times DSF \times ABS}{AT \times 10^6} \times \frac{CSF_o}{GIABS}$$

Where:

$$DSF_{ad} = \frac{ED_c \times EF_c \times SA_c \times AF_c}{BW_C} + \frac{ED_a \times EF_a \times SA_c \times AF_a}{BW_a} (mg\ kg^{-1})$$

c) Inhalation

$$LR_{inh} = \frac{C_{soil} \times EF \times ED \times ET}{AT \times PEF} \times IUR$$

1.3.2 Mutagenic risk

a) Ingestion

$$LR_{ing} = \frac{C_{soil} \times IFS_{ad}}{AT \times 10^6} \times CSF_o$$

Where:

$$IFS_{ad} = \frac{ED_{0-2} \times EF_{0-2} \times IRS_c \times 10}{BW_C} + \frac{ED_{2-6} \times EF_{2-6} \times IRS_c \times 3}{BW_c} + \frac{ED_{6-16} \times EF_{6-16} \times IRS_a \times 3}{BW_a} + \frac{ED_{16-30} \times EF_{16-30} \times IRS_a \times 1}{BW_a} (mg\ \ kg^{-1})$$

b) Dermal contact

$$LR_{der} = \frac{C_{soil} \times DSFM \times ABS}{AT \times 10^6} \times \frac{CSF_o}{GIABS}$$

Where:

$$DSFM_{ad} = \frac{ED_{0-2} \times EF_{0-2} \times SA_{0-2} \times AF_{0-2} \times 10}{BW_C} + \frac{ED_{2-6} \times EF_{2-6} \times SA_{2-6} \times AF_{2-6} \times 3}{BW_C} + \frac{ED_{6-16} \times EF_{6-16} \times SA_{6-16} \times AF_{6-16} \times 3}{BW_a} + \frac{ED_{16-30} \times EF_{16-30} \times SA_{16-30} \times AF_{16-30} \times 1}{BW_a} (mg\ kg^{-1})$$

c) Inhalation

$$LR_{inh} = \frac{C_{soil}}{AT \times PEF} \times [(ED_{0-2} \times EF_{0-2} \times ET_{0-2} IUR \times 10) + (ED_{2-6} \times EF_{2-6} \times ET_{2-6} \times IUR \times 3) + (ED_{6-16} \times EF_{6-16} \times ET_{6-16} \times IUR \times 3) + (ED_{16-30} \times EF_{16-30} \times ET_{16-30} \times IUR \times 1)] \times 10^{-3}$$

Annex II

Table 1 - Physical-chemical properties of PAHs.

Compound	Solubility ($mg\ L^{-1}$; 20-25 ºC)	Vapor pressure (mmHg; 20-25 ºC)	Henry's Law const. ($atm\ m^3\ mol^{-1}$)	Log K_{OW}	Log K_{OC} ($L\ kg^{-1}$)
NP	31[a];31.7[c]; 31.9[e]	8.5 x10^{-2c}	1.80 x10^{-2a} 4.83x10^{-4c}	3.34[e,f]; 3.36[c]	2.85[c]; 3.13[f] 3.19[a]
ACY	16.1[c,e]	8.9x10^{-1b} 9.1x10^{-4c}	1.13x10^{-5c} 1.14x10^{-3b}	3.93-4.1[c] 3.55[f];3.62[e];4.07[b]	3.34[f]; 3.75[c]
ACE	3.9 [a,c,d,e]	2.5x10^{-3c} 2.9x10^{-1b} 2.18 x10^{-3d}	1.84 x10^{-4a} 1.48x10^{-2b} 1.55x10^{-4c} 1.83x10^{-4d}	3.92[b;c,d,f] 4.00[e]	3.45[c] 3.70[a];3.71[f]
FLU	1.69[a]; 1.9[c] 1.68[d];1.8[e]	8.0x10^{-2b} 6.33x10^{-3}- 8.42x10^{-3c} 6.00 x10^{-4d}	9.62x10^{-5a} 6.34x10^{-5} – 1.0x10^{-4} 7.96x10^{-5c} 9.68 x10^{-5d}	4.18[b;c,d,f] 4.22[e]	3.69[c];3.96[a]; 3.97[f]
PHE	1.15[c] 0.977,1.18[d] 0.974[e]	1.6x10^{-2b} 1.12x10^{-4c} 1.20x10^{-4d}	2.33x10^{-5c} 4.23 x10^{-5d}	4.46,4.55[c]; 4.502[f]; 4.52[d]; 4.57[d,e]; 4.6[b]	3.82[c] 4.292[f]
ANT	0.0434[a] 0.043-0.075[c] 0.0436[d];0.047[e]	8.0x10^{-4b} 2.67x10^{-6c} 6.00x10^{-6d}	5.56 x10^{-5a} 1.93x10^{-5}-6.5x10^{-5} (3.54x10^{-5})[c] 5.57x10^{-5d}	4.45,4.55[c] 4.45[d]; 4.5[b]; 4.68[e,f]	4.21[a];4.3[c] 4.47[f]
FLA	0.26 [a c,d] 0.205[d]; 0.20[e]	1.2x10-3[b] 1.23x10^{-8c} 9.23 x10^{-6d}	8.86 x10^{-6a} 6.5x10^{-4b} 1.3x10^{-5} - 1.6x10^{-5} (1.44x10^{-5})[c] 1.93 x10^{-5d}	4.95[c]; 5.18[f]; 5.20[d,e]; 5.22[b]	4.62[c]; 4.74[a]; 4.97[f]
PYR	0.135 [a c,d] 0.13[d]; 0.125[e]	6.0x10-4[b] 2.45x10^{-6} – 4.59x10^{-6c} 4.50 x10^{-6d}	1.19 x10$^{-5 a}$ 1.1x10^{-}3[b] 1.1x10^{-5c} 1.69 x10^{-5d}	4.88-5.18[c] 4.96[f]; 4.98[e]; 5.0[d];5.18[b,d]	4.74[a] ; 4.75[f;] 4.84[c]
BAA	0.0094 [a c] 0.0090[d]; 0.0102[e]	2.8x10.5[b] 3.05x10^{-8} – 1.05x10^{-7c} 2.10 x10^{-7d}	1.2 x10^{-5a} 3.35x10^{-6c} 1.20 x10^{-5d}	5.61[b]; 5.7[c]; 5.91[d,e,f]	5.25[a]; 5.3[c]; 5.70[f]
CRY	0.0020[a,d] 0.002-0.0063[c] 0.00179[d];0.00165[e]	8.4x10^{-5b} 6.23x10^{-9c}	5.23 x10^{-6a} 5.23 x10^{-6d}	5.79,5.86[d] 5.7[c]; 5.81[e,f]	5.1[c];5.26[a]; 5.60[f]
BBF	0.0015[a,c,d] 0.00128[e]	5.0x10^{-7c}	6.57x10^{-7a} 5.1x10^{-5b} 1.11x10^{-4c}	5.78[d]; 6.12[b,e]; 6.124[f]; 6.2[c]	5.2[c]; 5.78[a]; 5.914[f]
BKF	0.0008[a,c,d] 0.00093[e]	1.3x10^{-8b} 2.0x10^{-9c}	5.84 x10^{-7a} 4.4x10^{-5b} 8.29x10^{-7c} 4.34 x10^{-7d}	6.11[d,e,f]; 6.2[c]; 6.84[b]	5.77[a]; 5.90[f]

Table 1 (cont) - Physical-chemical properties of PAHs.

Compound	Solubility (mg/L; 20-25 ºC)	Vapor pressure (mmHg; 20-25 ºC)	Henry's Law const. (atm m^3 mol^{-1})	Log Kow	Log Koc (L/kg)
BAP	0.00162[a c,d] 0.0038[d] 0.00154[e]	$7.3x10^{-7}$[b] $5.49x10^{-9}$[c]	$4.57x10^{-7}$ [a] $3.4x10^{-5}$[b] $1.13x10^{-6}$[c] $3.36 x10^{-7}$[d]	6.13[e,f]; 6.35[d]; 6.5[b]; 6.58[c];	5.77[a]; 5.92[f]; 6.34[c]
IND	0.00019[a,d] $2.2x10^{-5}$[c]	$1.3x10^{-8}$[b] $1.1x10^{-10}$[c]	$3.48 x10^{-7}$[a] $2.9x10^{-5}$[b] $1.6x10^{-6}$[c] $2.86 x10^{-7}$[d]	6.58[b,e]; 6.584[f]; 6.6[c]	6.2[c]; 6.29[a]; 6.374[f]
DBAH	0.00249 [a c] 0.0005[d] 0.00082[e]	$1.3x10^{-8}$[b] $1.0x10^{-10}$[c]	$1.41 x10^{-7}$[a] $7x10^{-6}$[b] $1.47x10^{-8}$[c]	6.5[b,c,e]; 6.55[f]; 6.69[c]; 6.75[d]	5.8-6.5[c]; 6.29[f]
BGHI	2.6x10-5[c] 0.00026[d]; 0.00014[e]	$1.0 x10^{-10}$[c]	$1.41x10^{-7}$[c] $2.66 x10^{-6}$[d]	6.22[e,f]; 6.7[c]; 6.9[d]	5.61[c]; 6.01[f]

[a] USEPA, 2011; [b]WHO, 2003; [c]CCME, 2010; [d] IARC, 2010; [e]EU, 2008; [f]Verbruggen, 2012

Table 2- Details of mild solvent extractions procedure found in literature.

Solvent	Bioassay	Compounds	Soil:solution (g/ml)	Extraction	Ref
BuOH	BD	PHE; PYR	2:10	30s (manual)	Bogan and Sullivan, 2003
BuOH	BD	NP	10:25	24h (shaker)	Kelsey and Alexander, 1997
BuOH	BD	10 PAHs	3:4.5	120s (vortex)	Latawiec and Reid, 2009;
BuOH	BD	PHE	3.5:35	12h (flat-bed shaker, 150 rpm)	Reid et al., 2000
BuOH	BD; EW	PHE, PYR	5 or 10:15	5s, 120s (vortex)	Liste and Alexander, 2002
BuOH; 50% MeOH or EtOH; 35% EtOHl	BD EW	PHE	10:25	2h (reciprocating shaker) no agitation	Kelsey et al., 1997
BuOH; MeOH; 50% EtOH	BD	15 PAHs	2.5 or 10:50	16h (end-over-end)	Thiele-Bruhn and Brümmer, 2004
BuOH; 50% or 100% PrOH	BD	12 PAHs	2:20	1, 10 or 60 min (end-over-end shaker)	Juhasz et al., 2005
Sequential leaching	BD	PYR	8:24	24 h, (tumble shaker, 100 rpm)	Macleod and Semple, 2003
BuOH	EW	PYR; BAA	5:10	2 min (manual)	Johnson et al., 2002
BuOH	EW	PYR	1:10	2h (reciprocating shaker, 125 rpm)	Sun and Li, 2005
BuOH	EW	PYR	2:25	20h (flat-bed rotary shaker, 150 rpm)	Khan et al., 2011
BuOH	EW; RT	5 PAHs	10:10 10:15	50s or 120s (vortex); 12h (end-over-end)	Gomez-Eyles et al., 2010
BuOH	EW; RT	12 PAHs	10:15	50s (vortex)	Gomez-Eyles et al., 2012
BuOH; PrOH; MeOH	EW; RT	ANT; FLA; PYR	1 or 2:25	5s (vortex)	Tang and Alexander, 1999
95% EtOH	EW	ANT, PYR, CRY, BaP	1:10 or 20	5-10s (vortex)	Tang et al., 2002
1% BuOH or MeOH; 50% MeOH; sequential leaching	EW	16 PAHs	2:20	24h (rock-n-roll)	Bergknut et al., 2007
BuOH; 50% MeOH	RT	NP, PHE, PYR, BAP	10:10	48h (reciprocating shaker, 100 rpm)	Tao et al., 2008
BuOH	TX	BAP	5:20	8s, vortex	Alexander and Alexander, 2000

BD-Biodegradation; EW-earthworm accumulation; RT-root accumulation; TX-toxicity test

Table 3 - Details of SPE extraction procedures found in literature.

Resin (g)	Bioassay	Compounds	Soil(g)	Resin (g)	Solution (ml)	Extraction	Resin extraction	Ref
XAD4	BD	NP	2.5	1	20 (0.01 M $CaCl_2$)	20 h (end-over-end, 60 rpm)	10 ml DCM (30 min, end-over-end)	Patterson et al., 2004
XAD4	EW	12 PAHs	10	1	125 (deionized water)	15 d (150 rpm)	20 ml DCM:Acetone 1:1 (2 min, US twice)	Bogan et al., 2005
XAD2	BD	20 PAHs	2	1.2	- (0.01 N $CaCl_2$)	Desorption kinetics	DCM	Hawthorne et al., 2001
Tenax	BD	PHE	-	0.2 (20/35)	- (inorganic salts)	Desorption kinetics (end-over-end)	ACN 16h	Braida et al., 2004
Tenax	BD	15 PAHs	5	1.1	50 (chelating agents)	Desorption kinetic, (orbital shaker, 100 rpm)	50 ml acetone, 48h	Li et al., 2005
Tenax	BD	12 PAHs	2.5	0.3	40 (0.01 M $CaCl_2$)	Desorption kinetics	n-hexane (3 times)	Bernhardt et al., 2013
Tenax	EW	PYR	1	0.2	10 (0.005 M $CaCl_2$)	Desorption kinetics (horizontal shaker, 120 rpm)	10 ml H:A 1:1(shaking 24h; twice)	Li et al., 2007
Tenax	EW	12 PAHs	1	1.5	70 (ultrapure water)	6h	30 ml n-hexane (few sec)	Ten Hulscher et al., 2003
Tenax	EW; RT	5 PAHs	1.4	1	70 (deionized water)	6h (end-over-end)	10 ml hexane (1h, US)	Gomez-Eyles et al., 2010
Tenax	Plant	16 PAHs	0.5 g	0.5 g	10 (deionized water)	24h (rotating shaker, 60 rpm)	10 ml H:A 1:1*2	Cofield et al., 2008

BD-Biodegradation; EW-earthworm accumulation; RT-root accumulation; TX-toxicity test

Table 4 - Details of solubilizing agents extraction procedures found in literature.

Solution	Bioassay	compound	Soil:solution (g/ml)	Solution	extraction	Ref
HPCD	BD; EW	PHE	1.5:25	50 mM	20h (orbital shaker, 100rpm)	Hickman and Reid, 2005; Doick et al., 2006; Doick et al., 2005
HPCD	BD	PHE; 14 PAHs	1.5:20	50 mM	24h, (orbital shaker, 100rpm)	Papadopoulos et al., 2007
HPCD	BD	PHE	1.25:25	50 mM	24h (orbital shaker, 100 rpm)	Rhodes et al., 2008a; Rhodes et al., 2008b; Rhodes et al., 2010
HPCD	BD	PHE	1.5:25	60mM	20h (orbital shaker, 100rpm)	Allan et al. 2006; Latawiec and Reid, 2009
HPCD	BD	4 or 10 PAHs	2.5:50	140gL^{-1}	Desorption kinetics (orbital shaker, 150 rpm)	Johnsen et al., 2006
HPCD	BD	6 PAHs	1:25	70 mM	Desorption kinetics (horizontal shaker, 150rpm)	Sabaté et al., 2006
HPCD	BD	16 PAHs	3:30	60 mM	2h, rotary shaker, 100rpm	Hickman et al., 2008
HPCD	BD	16/20 PAHs	1.25:25	50 mM	20h (orbital shaker, 100 rpm)	Stokes et al. 2005; Reid et al. 2000;
HPCD	BD	12 PAHs	1.25:25	40mM	20h (150rpm)	Juhasz et al., 2005
HPCD	BD	12 PAHs	2.5:50	50 mM	Desorption kinetics	Bernhardt et al., 2013
HPCD	EW	PYR	2:25	50 mM	20h (flat-bed rotary shaker, 150 rpm)	Khan et al., 2011
HPCD	EW;RT	5 PAHs	1.5:25	60mM	20h (orbital shaker, 200rpm)	Gomez-Eyles et al. 2010; Gomez-Eyles et al. 2012
HPCD	EW	16 PAHs	2:20	50 mM	24h (rock-n-roll)	Bergknut et al., 2007
HPCD	TX	PHE	1.25:25	50 mM	20h (orbital shaker, 100 rpm)	Brown et al., 2010
Brij 700	BD	10 PAHs	3:30	-	16h (rotary shaker, 100rpm)	Latawiec and Reid, 2009
Genapol UDD 88; SynperonicLF7RA 30	BD	15	1:20	5 mg/L	16h (end-over-end)	Thiele-Bruhn and Brümmer, 2004
Tween 80	EW	16	2:20	3mM	24h (rock-n-roll)	Bergknut et al., 2007

BD-Biodegradation; EW-earthworm accumulation; RT-root accumulation; TX-toxicity test

Table 5 - Details of SPME extraction procedures found in literature.

Bioassay	Compounds	Soil:solution (m/ml)	Solution	Fiber details	Nº fibers	Exposition	Ref
EW; RT	12 PAHs	2:6	0.01 M $CaCl_2$	PDMS (110 µm glass core diameter, 28.5 µm thick coating)	4x5cm	28 days, 20ºC, rock and roll shaker	Gomez-Eyles et al., 2012
EW	15 PAHs	2:5	-	PDMS (40um, total diameter 250 um)	10 cm	21d in rock-n- roll	Bergknut et al., 2007
EW;TX	13 PAHs	1 or 2:5	0.01M $CaCl_2$	PDMS (110µm glass core diameter, 28.5 µm thick coating)	1 or 2x5 cm	Rock and Roller, 672h	Jonker et al., 2007
TX	PYR	2	-	PDMS (110µm glass core diameter, 28.5 µm thick coating)	5cm	Rock and Roller, 48h	Styrishave et al., 2008
TX	PHE	0.5 or 1:2	-	PDMS (430µm glass core diameter, 35 µm thick coating)	1x0.5cm	200 rpm, room temp, 3 d	Yang et al., 2009

BD-Biodegradation; EW-earthworm accumulation; RT-root accumulation; TX-toxicity test

Annex III

Table 1 - Descriptive statistics of PTEs concentrations in Lisbon (LIS) and Viseu (VIS) urban soils.

Element		n	Mean	Median	Min.	Max.	1st Q	3rd Q	Std. Dev.	RSD (%)
Ag (mg kg^{-1})	LIS	27	0.30	0.20	0.1	1.7	0.1	0.3	0.33	110
	VIS	7	0.33	0.20	0.2	0.8	0.2	0.5	0.24	73
Al (%)	LIS	51	1.2	0.83	0.21	3.1	0.64	1.7	0.84	70
	VIS	14	2.5	2.6	1.7	3.3	2.0	2.9	0.50	20
As (mg kg^{-1})	LIS	51	5.3	4.4	0.50	29	2.7	6.3	4.6	87
	VIS	14	30	24	17	58	18	46	15	50
Ba (mg kg^{-1})	LIS	51	107	72	14	429	47	149	88	82
	VIS	14	79	80	43	130	64	92	23	29
Bi (mg kg^{-1})	LIS	46	0.25	0.2	0.1	1.0	0.2	0.3	0.15	60
	VIS	14	0.76	0.70	0.3	1.3	0.5	1.1	0.33	43
Ca (%)	LIS	51	3.8	3.1	0.17	13	1.7	4.9	2.8	74
	VIS	14	0.42	0.44	0.20	0.61	0.38	0.46	0.14	33
Cd (mg kg^{-1})	LIS	38	0.21	0.10	0.10	0.60	0.20	0.30	0.12	57
	VIS	11	0.15	0.10	0.10	0.50	0.1	0.2	0.12	80
Co (mg kg^{-1})	LIS	51	13	6.8	0.6	49	4.2	18	13	100
	VIS	14	5.9	5.9	3.1	7.9	5.3	7.0	1.3	22
Cr (mg kg^{-1})	LIS	51	38	16	1.0	172	9.0	44	44	116
	VIS	14	11	10	6.0	17	9.0	13	2.7	25
Cu (mg kg^{-1})	LIS	51	37	29	3.5	143	18	52	26	70
	VIS	14	33	27	6.1	78	24	40	20	61
Fe (%)	LIS	51	2.1	1.6	0.18	5.9	1.0	3.0	1.5	71
	VIS	14	2.4	2.5	1.5	3.3	2.1	2.7	0.49	20
Ga (mg kg^{-1})	LIS	49	4.0	3.0	1.0	10	2.0	6.0	2.7	68
	VIS	14	11	11	6.0	14	9.5	12	2.2	20
K (%)	LIS	51	0.17	0.17	0.03	0.42	0.12	0.22	0.08	47
	VIS	14	0.51	0.51	0.25	0.82	0.44	0.56	0.13	25
La (mg kg^{-1})	LIS	51	16	11	2.0	41	10	21	9.9	62
	VIS	14	24	22	15	47	18	28	8.8	37
Mg (%)	LIS	51	0.53	0.34	0.02	2.0	0.18	0.55	0.52	98
	VIS	14	0.51	0.51	0.25	0.82	0.44	0.56	0.10	20
Mn (mg kg^{-1})	LIS	51	337	218	11	1193	126	471	298	88
	VIS	14	360	368	186	500	289	422	92	26
Mo (mg kg^{-1})	LIS	51	0.67	0.6	0.1	1.9	0.4	0.9	0.34	51
	VIS	14	0.61	0.6	0.4	0.8	0.5	0.73	0.15	25
Na (%)	LIS	51	0.04	0.02	0.01	0.19	0.02	0.05	0.04	100
	VIS	14	0.01	0.01	0.01	0.03	0.01	0.01	0.01	100
Ni (mg kg^{-1})	LIS	51	43	20	2.0	209	12	44	52	121
	VIS	14	5.0	4.5	3.3	9.7	3.6	5.2	2.1	42
P (%)	LIS	51	0.11	0.10	0.02	0.26	0.05	0.16	0.06	55
	VIS	14	0.08	0.07	0.03	0.15	0.04	0.11	0.04	50

Table 1 (cont) - Descriptive statistics of PTEs concentrations in Lisbon (LIS) and Viseu (VIS) urban soils.

Element		n	Mean	Median	Min.	Max.	1st Q	3rd Q	Std. Dev.	RSD (%)
Pb (mg kg^{-1})	LIS	51	89	62	4.8	561	27	108	98	110
	VIS	14	106	46	13	817	21	78	207	195
S (%)	LIS	16	0.11	0.12	0.06	0.15	0.09	1.3	0.03	27
	VIS	1	-	-	0.07	0.07	-	-	-	-
Sb (mg kg^{-1})	LIS	50	0.84	0.70	0.10	3.5	0.48	1.3	0.64	76
	VIS	14	0.78	0.4	0.1	5.6	0.1	0.73	1.4	179
Sc (mg kg^{-1})	LIS	50	2.4	1.3	0.3	9.8	0.70	3.2	2.4	100
	VIS	14	6.1	6.4	2.8	8.7	5.3	7.1	1.5	25
Se (mg kg^{-1})	LIS	9	0.79	0.8	0.5	1.3	0.65	0.8	0.22	28
	VIS	4	0.55	0.55	0.5	0.6	0.5	0.6	0.06	11
Sr (mg kg^{-1})	LIS	51	76	56	4.0	226	26	120	61	80
	VIS	14	12	11	5.0	25	8.8	13	5.9	49
Th (mg kg^{-1})	LIS	51	1.8	1.6	0.60	4.9	0.9	2.4	0.97	54
	VIS	14	9.8	9.4	5.4	16	6.9	13	3.4	35
Ti (%)	LIS	51	0.08	0.03	0.003	0.35	0.01	0.09	0.10	125
	VIS	14	0.18	0.19	0.08	0.26	0.17	0.21	0.05	28
Tl (mg kg^{-1})	LIS	22	0.14	0.1	0.10	0.30	0.1	0.20	0.06	43
	VIS	14	0.88	0.85	0.7	1.1	0.78	1.0	0.14	16
U (mg kg^{-1})	LIS	51	0.7	0.70	0.20	1.3	0.50	0.90	0.23	33
	VIS	14	8.3	8.9	5.9	11	6.9	9.3	1.5	18
V (mg kg^{-1})	LIS	50	48	27	2.0	178	12	83	49	102
	VIS	14	37	37	19	48	32	44	8.6	23
W (mg kg^{-1})	LIS	18	0.16	0.2	0.1	1.8	0.1	0.2	0.39	244
	VIS	14	0.95	1.0	0.2	2.2	0.3	1.3	0.48	51
Zn (mg kg^{-1})	LIS	51	97	88	7.0	269	59	132	53	55
	VIS	14	88	80	47	190	63	111	38	43
Hg (mg kg^{-1})	LIS	51	0.36	0.18	0.01	3.8	0.08	0.34	0.61	169
	VIS	14	0.26	0.11	0.02	1.6	0.05	0.27	0.41	158

1º Q – 1º Quartil; 3ºQ – 3º Quartil

Table 2 - Range of isomer ratios observed in the present study and in other studies around the world. Mean values, when available, are presented between brackets.

Local	Matrix	ANT/178	FLA/202	BAA/228	IND/278	Ref.
Lisbon, Portugal	Urban soils	0.11-0.34 (0.18)	0.50-0.57 (0.53)	0.29-0.53 (0.45)	0.26-0.56 (0.47)	This study
Viseu, Portugal	Urban soils	0.01-0.23 (0.14)	0.33-0.63 (0.53)	0.23-0.52 (0.42)	0.27-0.46 (0.40)	This study
Beijing, China	Urban soils	-	0.5-1.0 (0.59)	-	-	Liu et al., 2010a;
Shangai, China	Urban soils	0.01-0.26	- 0.11-0.77	0.3-0.6	0.44-0.58 0.3-0.56	Peng et al., 2011 Liu et al., 2010b
Harbin, China	Urban/ Rural soils	0.07-0.19 urb 0.07-0.12 rur	-	0.42-0.49urb 0.17-0.49rur	-	Ma et al., 2009
Switzerland	Soils	0.003-0.11 (0.02)	0.53-0.72 (0.58)	0.27-0.58 (0.40)	0.36-0.55 (0.49)	Brandli et al., 2008
Lisbon, Portugal	Aerosol particles	-	0.48-0.58	0.41-0.52	0.31-0.73	Oliveira et al., 2011
United Kingdom	Ambient air	<0.1	>0.5	0.2-0.4	0.4-0.5	Katsoyiannis et al., 2011
-	-	<0.1 petrogenic >0.1 pyrogenic	<0.4 petrogenic 0-4-0.5 liquid fossil fuel >0.5 coal and biomass	<0.2 petrogenic >0.2 pyrogenic	<0.2 petrogenic 0-2-0.5 liquid fossil fuel >0.5 coal and biomass	Yunker et al., 2002 Brandli et al., 2008

Table 3 - Spearman correlation matrix among PAHs, PCBs, general parameters and PTEs in Lisbon (dark grey) and Viseu (light grey).

	∑PAHs	∑PCBs	pH_{Ca}	OC	OM	CEC	Sand	Silt	Clay	As	Co	Cr	Cu	Ni	Pb	Zn	Hg
∑PAHs		**-**	**-**	0.65	0.58	-	-	-	**-0.67**	0.54	-	-	-	-	**0.88**	**0.76**	**0.83**
∑PCBs	**0.61**		-	-	-	-	-	-	-	-	-	-	-	-	0.60	-	-
pH_{Ca}	-	**-**		-	-	-	-	-	-	0.60	-	-	-	**-**	-	-	**-**
OC	**0.47**	**0.61**	**-0.46**		**0.87**	**0.72**	-	-	-0.57	-	-	-	-	**-**	**0.68**	-	0.54
OM	0.31	**0.49**	**-0.46**	**0.89**		**0.75**	-	-	-0.60	-	-	-	-	-	0.64	-	-
CEC	**-**	**0.46**	-0.34	**0.77**	**0.91**		-	-	-	-	-	-	-	-	0.64	-	-
Sand	**-**	-	**-**	**-0.53**	**-0.64**	**-0.74**		**-0.79**	-0.54	-	-	-	**-**	-	-	**-**	-
Silt	0.29	-	**-**	**0.47**	**0.49**	**0.57**	**-0.87**		**-**	-	-	-	**-**	-	-	**-**	-
Clay	**-**	**-**	**-**	**0.40**	**0.58**	**0.72**	**-0.83**	**0.51**		-	-	-	-	-	-0.60	**-0.68**	-
As	0.35	-	0.31	-	**-**	**-**	**-**	**-**	**-**		-	-	-	-	-	-	0.57
Co	**-**	-	**-**	**0.47**	**0.64**	**0.71**	**-0.60**	**0.39**	**0.71**	**-**		0.53	-	**0.89**	-	-	**-**
Cr	**-**	0.31	**-**	**0.46**	**0.64**	**0.69**	**-0.60**	**0.39**	**0.69**	**-**	**0.96**		-	-	-	-	-
Cu	**0.63**	**0.59**	**.-367**	**0.59**	**0.59**	**-**	**-0.37**	**0.39**	**-**	**-**	**0.52**	**0.59**		-	**-**	-	-
Ni	**-**	0.30	**-**	**0.50**	**0.67**	**0.65**	**-0.58**	**0.39**	**0.65**	**-**	**0.93**	**0.94**	**0.61**		-	-	0.54
Pb	**0.87**	**0.55**	-	**0.47**	0.32	-	**-**	0.32	**-**	**0.42**	-	-	**0.69**	-		**-**	**0.83**
Zn	**0.70**	**0.69**	-	**0.62**	**0.56**	-	**-0.37**	**0.44**	-	0.30	**0.36**	**0.43**	**0.84**	**0.51**	**0.78**		0.58
Hg	**0.80**	**0.49**	**-**	**0.52**	**0.37**	**-**	-	-	-	0.36	**-**	**-**	**0.70**	**-**	**0.85**	**0.71**	

Numbers in bold means that correlation is significant at the 0.01 level (2-tailed), for the others correlation is significant at the 0.05 level (2-tailed)

Table 4 - Spearman correlation matrix among PAHs, PCBs and PTEs after normalization to OC content, in Lisbon (dark grey) and Viseu (light grey).

	∑PAHs	∑PCBs	As	Co	Cr	Cu	Ni	Pb	Zn	Hg
∑PAHs		-	-	-	-	-	-	**.79**	**-**	**.77**
∑PCBs	**.47**		-	-	-	-	-	-	-	-
As	.34	-		**.72**	**.73**	**.68**	**.74**	-	**.79**	-
Co	-	-	-		**.94**	**.68**	**.99**	-	**.99**	-
Cr	-	-	-	**.94**		-	**.93**	-	**.84**	-
Cu	**.52**	**.36**	-	**.30**	**.39**		**.67**	-	-	-
Ni	-	-	-	**.91**	**.93**	**.39**		-	**.87**	-
Pb	**.85**	**.39**	**.40**	**-**	**-**	**.64**	-		-	**.80**
Zn	**.60**	**.41**	**.43**	**-**	**-**	**.83**	**-**	**.73**		-
Hg	**.78**	.29	.32	**-**	**-**	**.63**	**-**	**.84**	**.61**	

Numbers in bold means that correlation is significant at the 0.01 level (2-tailed), for the others correlation is significant at the 0.05 level (2-tailed)

Table 5 - Descriptive statistics of PAHs concentrations in Lisbon and Viseu soils, expressed in µg BAPeq kg^{-1}.

Compounds	TEF[a]	Lisbon				Viseu			
		mean	median	min	max	mean	median	min	max
NP	0.001	0.008	0.004	bdl	0.12	0.002	0.001	0.0003	0.005
ACY	0.001	0.009	0.002	bdl	0.17	0.001	0.001	0.0001	0.008
ACE	0.001	0.004	0.002	bdl	0.048	0.001	0.0006	bdl	0.004
FLU	0.001	0.004	0.002	bdl	0.039	0.002	0.001	0.0002	0.01
PHE	0.001	0.09	0.029	bdl	0.93	0.02	0.005	0.0008	0.07
ANT	0.01	0.21	0.07	bdl	2.6	0.03	0.01	Bdl	0.16
FLA	0.001	0.24	0.07	bdl	3.7	0.03	0.01	0.0004	0.13
PYR	0.001	0.22	0.06	0.001	3.5	0.02	0.01	0.0005	0.11
BAA	0.1	11	3.1	bdl	146	1.2	0.36	Bdl	6.3
CRY	0.01	1.3	0.40	bdl	18	0.13	0.07	0.003	0.58
BBF	0.1	15	4.4	bdl	244	1.8	0.87	0.03	8.9
BKF	0.1	11	2.9	bdl	182	1.0	0.45	0.02	5.9
BAP	1	144	36	bdl	2170	16	7.4	0.30	80
IND	0.1	13	3.9	bdl	190	0.97	0.45	0.020	4.5
DBAH	1	31	7.8	bdl	436	3.2	1.5	0.25	20
BGHI	0.01	1.3	0.47	bdl	19	0.14	0.08	0.003	0.68
∑BAPeq (16PAHs)	-	229	61	0.18	3416	24	11	0.63	127
∑BAPeq (7PAHs)	-	226	60	0.12	3386	24	11	0.62	126

[a]Tsai et al., 2004

Table 6 - Statistics of ingestion (Ing), dermal (Derm), inhalation (Inh) and total cancer risks of HOCs in both cities, considering an occupational land use.

	Lisbon				Viseu			
	Ing	**Derm**	**Inh**	**Total**	**Ing**	**Derm**	**Inh**	**Total**
PAHs								
Mean	5.2E-07	4.5E-07	1.4E-11	**9.7E-07**	5.6E-08	4.8E-08	1.4E-12	**1.0E-07**
Med	1.4E-07	1.2E-07	3.6E-12	**2.6E-07**	2.6E-08	2.2E-08	6.7E-13	**4.8E-08**
Min	4.2E-10	3.6E-10	1.1E-14	**7.9E-10**	1.4E-09	1.2E-09	3.7E-14	**2.7E-09**
Max	7.8E-06	6.7E-06	2.0E-10	**1.5E-05**	2.9E-07	2.5E-07	7.6E-12	**5.4E-07**
UCL (95%)	1.3E-06	1.1E-06	3.3E-11	**2.4E-06**	1.1E-07	9.3E-08	2.8E-12	**2.0E-07**
PCBs								
Mean	4.4E-09	4.1E-09	2.2E-13	**8.5E-09**	2.9E-09	2.7E-09	1.4E-13	**5.6E-09**
Med	2.9E-09	2.9E-09	1.4E-13	**5.7E-09**	1.4E-09	1.3E-09	6.9E-14	**2.7E-09**
Min	1.5E-10	1.5E-10	7.7E-15	**3.0E-10**	1.2E-10	1.1E-10	5.7E-15	**2.2E-10**
Max	2.2E-08	2.0E-08	1.1E-12	**4.2E-08**	9.3E-09	8.6E-09	4.6E-13	**1.8E-08**
UCL (95%)	5.6E-09	5.2E-09	2.8E-13	**1.1E-08**	5.3E-09	4.9E-09	2.6E-13	**1.0E-08**

Table 7 - Concentrations of PAHs and results of ingestion (Ing), dermal (Derm), inhalation (Inh) and total cancer and mutagenic risks in both cities, considering a recreational land use.

		Cancer				Mutagenic			
	C_{soil} ($\mu g\ kg^{-1}$)	Ing	Derm	Inh	Total	Ing	Derm	Inh	Total
Lisbon									
Mean	197	1.7E-07	6.9E-08	3.9E-13	**2.4E-07**	7.2E-07	2.8E-07	9.9E-13	**9.9E-07**
Med	46	3.9E-08	1.6E-08	9.0E-14	**5.5E-08**	1.7E-07	6.4E-08	2.3E-13	**2.3E-07**
Min	0.18	1.6E-10	6.4E-11	3.6E-16	**2.2E-10**	6.7E-10	2.6E-10	9.2E-16	**9.3E-10**
Max	3416	2.9E-06	1.2E-06	6.7E-12	**4.1E-06**	1.2E-05	4.8E-06	1.7E-11	**1.7E-05**
UCL (95%)	362	3.1E-07	1.3E-07	7.2E-13	**4.3E-07**	1.3E-06	5.1E-07	1.8E-12	**1.8E-06**
Viseu									
Mean	26	2.2E-08	8.9E-09	5.1E-14	**3.1E-08**	9.3E-08	3.6E-08	1.3E-13	**1.3E-07**
Med	12	1.0E-08	4.2E-09	2.4E-14	**1.4E-08**	4.4E-08	1.7E-08	6.0E-14	**6.0E-08**
Min	2.9	2.4E-09	1.0E-09	5.7E-15	**3.4E-09**	1.0E-08	4.0E-09	1.4E-14	**1.4E-08**
Max	127	1.1E-07	4.4E-08	2.5E-13	**1.5E-07**	4.6E-07	1.8E-07	6.4E-13	**6.4E-07**
UCL (95%)	56	4.8E-08	2.0E-08	1.1E-13	**6.7E-08**	2.0E-07	7.8E-08	2.8E-13	**2.8E-07**

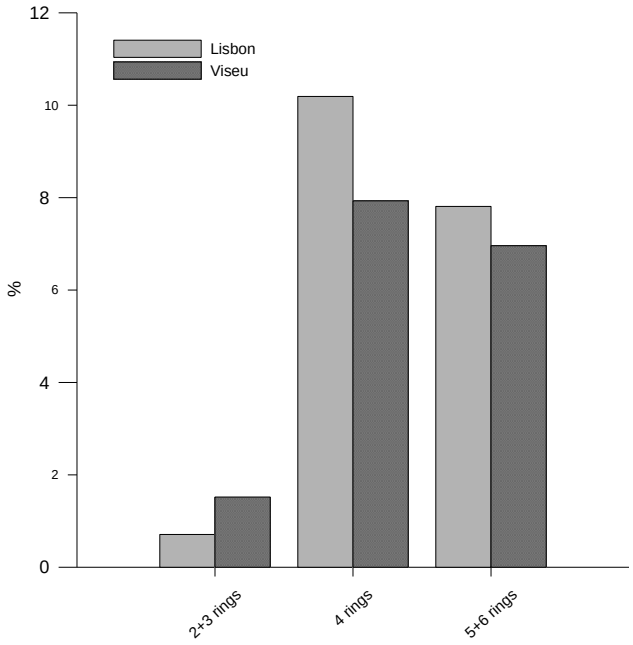


Figure 1 - Median percentage of PAHs groups observed for the two cities**.**

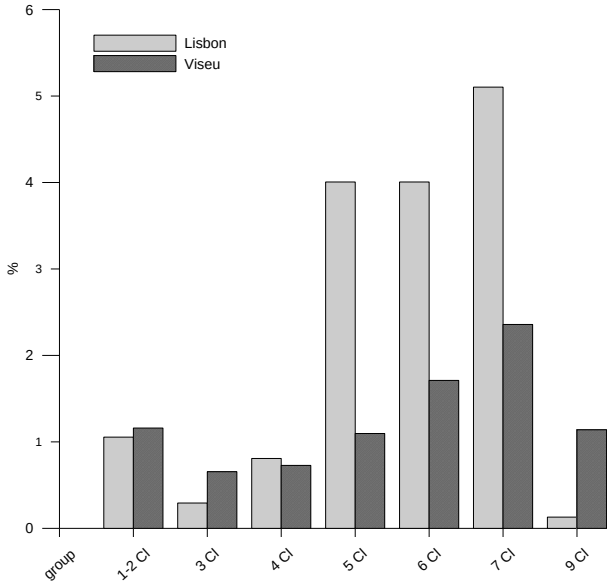


Figure 2 - Median percentage of PCBs groups observed for the two cities.

Annex IV

Table 1 - Results of the soil's general parameters determined in the 2nd sampling campaign of Lisbon: pH, total carbon (TC), organic carbon (OC), organic matter (OM), cation exchange capacity (CEC) and particle size (sand, silt and clay).

Parameter	**Mean**	**Median**	**Min**	**Max**
pH ($CaCl_2$)	6.7	6.8	5.5	7.2
TC (%)	5.0	4.0	0.69	24
OC (%)	2.6	2.3	0.57	8.6
OM (%)	7.3	6.0	0.91	41
CEC (cmol kg^{-1})	18	18	2.1	48
Sand (%)	64	64	29	96
Silt (%)	24	24	2.8	48
Clay (%)	12	11	0.91	27

Table 2 - Descriptive statistics of PTEs concentrations in Lisbon urban soils in the 2nd sampling campaign.

Element	**Mean**	**Median**	**Min**	**Max**	**RSD (%)**
Al (%)	1.2	1.1	0.25	4.3	59
Ca (%)	4.2	3.8	0.10	12	64
Fe (%)	2.1	1.9	0.52	7.3	57
As (mg kg^{-1})	4.3	4.4	<0.10	9.3	56
Co (mg kg^{-1})	8.4	5.7	0.80	43	97
Cr (mg kg^{-1})	42	31	7.4	201	91
Cu (mg kg^{-1})	55	44	7.0	258	83
Hg (mg kg^{-1})	0.56	0.24	0.03	3.6	134
Ni (mg kg^{-1})	33	22	4.3	194	110
Pb (mg kg^{-1})	98	77	4.7	305	83
Zn (mg kg^{-1})	138	119	11	540	70

Table 3 - Descriptive statistics of PTEs concentrations in Lisbon street dusts.

Element	**Mean**	**Median**	**Min**	**Max**	**RSD (%)**
Al (%)	0.34	0.25	0.08	3.88	158
Ca (%)	11	9.8	0.74	31	59
Fe (%)	0.66	0.58	0.15	2.0	59
As (mg kg^{-1})	3.05	2.90	1.05	8.07	49
Co (mg kg^{-1})	3.57	2.71	0.58	21.2	95
Cr (mg kg^{-1})	16.3	8.44	2.40	219	200
Cu (mg kg^{-1})	118	41.3	6.81	978	158
Hg (mg kg^{-1})	0.13	0.06	0.01	0.63	122
Ni (mg kg^{-1})	13.4	10.0	1.88	66.2	89
Pb (mg kg^{-1})	95.8	62.8	6.99	634	136
Zn (mg kg^{-1})	324	178	39.1	2892	151

Table 4 - Spearman correlation matrix among PAHs and PTEs in Lisbon dusts (dark grey) and soils (light gray).

	∑PAHs	2+3 rings	4 rings	5+6 rings	Al	As	Ca	Co	Cr	Cu	Fe	Hg	Ni	Pb	Zn
∑PAHs		**.96**	**1.00**	**.99**	-	**.39**	**.59**	-	-	**.60**	-	**.68**	-	**.77**	**.52**
2+3 rings	**.94**		**.95**	**.92**	-	**.44**	**.59**	-	-	**.63**	-	**.66**	-	**.77**	**.55**
4 rings	**.98**	**.94**		**.98**	-	**.39**	**.57**	-	-	**.607**	-	**.69**	-	**.77**	**.51**
5+6 rings	**.98**	**.89**	**.94**		-	**.38**	**.60**	-	-	**.59**	-	**.67**	-	**.75**	**.52**
Al	-	-	-	.29		-	**-**	**.82**	**.83**	.35	**.89**	-	**.80**	-	.30
As	**.41**	-	**.38**	**.42**	.34		**.43**	**-.38**	-.32	-	-	-	-.33	**.40**	-
Ca	-	-	-	-	-	-		-	-	**.37**	-	**.43**	-	**.51**	**.40**
Co	**.38**	.30	.31	**.41**	**.72**	**.48**	-		**.92**	**.50**	**.93**	**-**	**.96**	-	**.45**
Cr	**.36**	.32	.29	**.38**	**.64**	**.52**	-	**.82**		**.59**	**.91**	**-**	**.94**	-	**.50**
Cu	**.61**	**.57**	**.53**	**.66**	**.49**	**.43**	**.39**	**.62**	**.72**		**.55**	**.66**	**.63**	**.77**	**.72**
Fe	**.43**	.36	.36	**.46**	**.77**	**.55**	-	**.83**	**.87**	**.71**		**-**	**.94**	-	**.46**
Hg	**.48**	**.41**	**.46**	**.50**	**.41**	**.61**	-	**.47**	**.41**	**.55**	**.49**		**.38**	**.88**	**.54**
Ni	**.40**	.36	.32	**.44**	**.70**	.34	.35	**.86**	**.78**	**.78**	**.82**	**.49**		.35	**.55**
Pb	**.70**	**.66**	**.65**	**.72**	**.52**	**.56**	-	**.51**	**.64**	**.76**	**.65**	**.59**	**.60**		**.71**
Zn	**.41**	**.39**	.36	**.43**	.31	**.45**	-	**.62**	**.73**	**.76**	**.61**	**.58**	**.62**	**.62**	

Numbers in bold means that correlation is significant at the 0.01 level (2-tailed), for the others correlation is significant at the 0.05 level (2-tailed)

Table 5 – Canadian interim sediment quality guidelines (ISQGs) for the protection of aquatic life and probable effect level (PEL) for both freshwater and marine sediments (μg kg^{-1}).

Compound	ISQGs		PEL	
	Freshwater	Marine	Freshwater	Marine
NP	34.6	34.6	391	391
ACE	6.71	6.71	88.9	88.9
ACY	5.87	5.87	128	128
FLU	21.2	21.2	144	144
PHE	41.9	86.7	875	1398
ANT	46.9	46.9	245	245
FLA	111	113	2355	1494
PYR	53	153	875	1398
BAA	31.7	74.8	385	693
CRY	57.1	108	862	846
BAP	31.9	88.8	782	763
DahA	6.22	6.22	135	135

Table 6 - Descriptive statistics of PAHs concentrations in street dusts and soils from Lisbon, expressed in µg BAPeq kg^{-1}.

Compounds	TEF[a]	Dusts				Soils			
		mean	median	min	max	mean	median	min	max
NP	0.001	0.008	0.007	0.001	0.047	0.010	0.004	0.000	0.115
ACY	0.001	0.010	0.003	0.000	0.230	0.007	0.002	0.000	0.116
ACE	0.001	0.013	0.004	0.000	0.223	0.009	0.002	0.000	0.204
FLU	0.001	0.016	0.005	0.001	0.266	0.008	0.002	0.000	0.181
PHE	0.001	0.269	0.063	0.006	5.88	0.216	0.046	0.001	4.733
ANT	0.01	0.414	0.080	0.008	8.73	0.403	0.107	0.005	7.380
FLA	0.001	0.710	0.077	0.007	18.0	0.796	0.087	0.002	18.3
PYR	0.001	0.576	0.095	0.007	14.7	0.621	0.079	0.002	13.4
BAA	0.1	22.329	3.048	0.354	474	31.079	4.777	0.111	541
CRY	0.01	3.825	0.652	0.057	87.5	3.363	0.617	0.018	60.6
BBF	0.1	29.507	4.783	0.803	533	42.185	8.243	0.144	759
BKF	0.1	30.889	3.026	0.263	844	26.248	4.298	0.150	444
BAP	1	236.094	40.433	4.472	5,009	292.460	63.255	1.325	4,500
IND	0.1	24.063	3.725	0.426	549	25.444	5.868	0.193	354
DBAH	1	59.686	8.423	0.776	1,460	61.769	12.001	0.250	992
BGHI	0.01	0.008	0.007	0.001	0.047	2.403	0.639	0.018	32.3
∑BAPeq	-	409	66.0	9.35	8,994	485	107	2.69	7,683

[a]Tsai et al., 2004

Table 7 - Statistical data of total cancer and mutagenic risks for different land uses of soil samples.

	Residential		Worker	Recreational	
	Cancer	**Mutagenic**	**Cancer**	**Cancer**	**Mutagenic**
UCL	2.18E-05	8.10E-05	5.78E-06	3.53E-07	1.48E-06
med	1.72E-06	7.26E-06	4.57E-07	1.22E-07	5.15E-07
mean	7.80E-06	3.28E-05	2.07E-06	2.51E-07	1.05E-06
90%	9.78E-06	4.12E-05	2.59E-06	6.86E-07	2.88E-06
min	4.33E-08	1.82E-07	1.15E-08	3.22E-09	1.35E-08
máx	1.24E-04	5.20E-04	3.28E-05	1.56E-06	6.56E-06

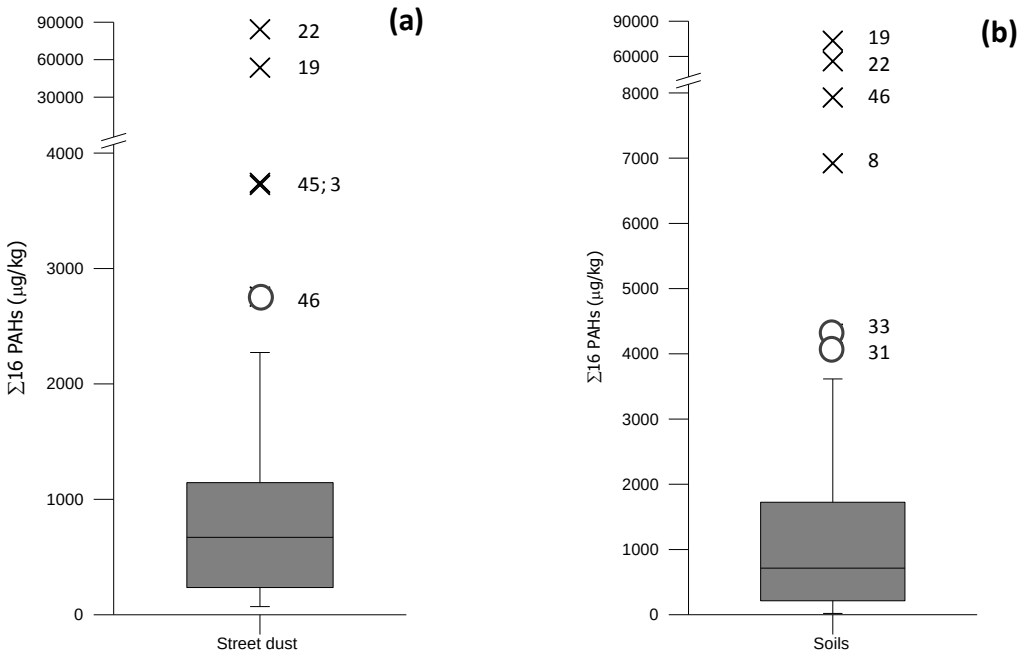


Figure 1 - Boxplots showing the variation of PAHs concentrations in dusts (a) and soils (b). Boxes define the interquartile range and the line is the median. Outliers are defined as values between 1.5 and 3 box lengths (o) and extreme values as more than 3 box lengths (x).

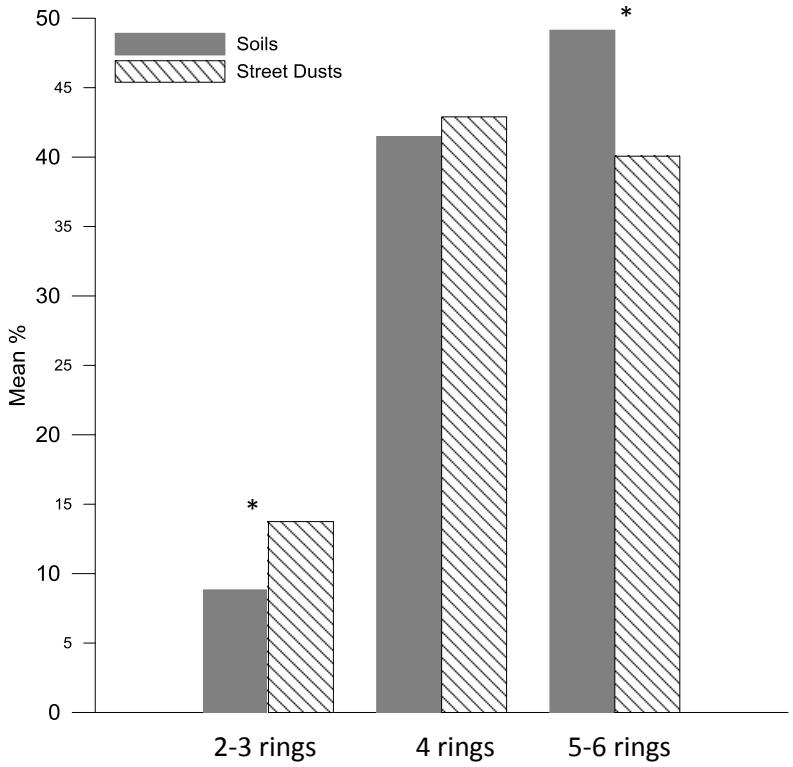


Figure 2 - Mean percentages of groups of PAHs in soils and dusts. Statistical significant differences between the two matrixes are signed: * for $p<0.05$ and ** for $p<0.01$.

Annex V

Table 1 - Descriptive statistics of PAHs concentrations (μg kg^{-1}) in Lisbon urban soils for the 1st and 2nd sampling campaign and the final dataset.

	Mean			Median			Min			Máx		
	1st	2nd	Final	1st	2nd	Final	1st	2nd	Final	1st	2nd	Final
NP	7.9	10.0	8.4	4.4	3.9	4.0	bdl	bdl	bdl	122	115	122
ACY	8.9	6.7	7.1	2.2	1.7	2.0	bdl	bdl	bdl	171	116	171
ACE	3.9	9.1	6.2	1.6	1.8	1.6	bdl	bdl	bdl	47.6	204	204
FLU	4.0	8.4	5.9	1.6	2.2	1.8	bdl	bdl	bdl	39	181	181
PHE	92	216	147	29	46	31	bdl	1.5	bdl	929	4,733	4,733
ANT	21	40	29	6.8	11	7.0	bdl	0.46	bdl	261	738	738
FLA	244	796	506	71	87	74	0.04	1.6	0.04	3670	18,259	18,259
PYR	223	621	408	60	78	66	1.1	1.7	1.1	3,550	13,429	13,429
BAA	108	311	208	31	48	40	bdl	1.1	bdl	1,462	5,406	5,406
CRY	127	336	228	39	62	44	bdl	1.8	bdl	1,813	6,066	6,066
BBF	155	422	288	44	82	65	bdl	1.4	bdl	2,441	7,591	7,591
BKF	111	262	170	29	43	32	bdl	1.5	bdl	1,825	4,439	3,819
BAP	144	292	212	36	63	46	bdl	1.3	bdl	2,170	4,500	4,500
IND	132	254	190	39	59	43	bdl	1.9	bdl	1,896	3,543	3,543
DBAH	31	62	45	7.7	12	9.5	bdl	bdl	bdl	436	992	992
BGHI	133	240	181	47	64	54	bdl	1.8	bdl	1,873	3,235	3,235
∑16PAHs	1,544	3,888	2,645	456	714	559	6.3	20	6.3	22,670	73,395	73,395

Table 2 - Median, mean concentration and range of PAHs (μg kg^{-1}) in urban soils around the world.

	Median	Mean	Min	Max	Ref.
Lisbon, Portugal	559	2,645	6.3	73,395	This study
	456	1,544	6.3	22,670	Cachada et al., 2012a
Viseu, Portugal	83	169	6.0	790	Cachada et al., 2012a
Estarreja, Portugal	98	-	27	2,016	Cachada et al., 2012b
Glasgow, Scotland	8,337[a]	11,930[a]	1,487[a]	51,822[a]	Morillo et al., 2007
Torino, Italy	704[a]	1,990[a]	148[a]	23,500[a]	Morillo et al., 2007
Ljubljana, Slovenia	791[a]	989[a]	218[a]	4,488[a]	Morillo et al., 2007
Terragona, Spain	-	438	42	1472	Nadal et al., 2007
Beijing, China	688	1,228	93	13,141	Peng et al., 2011
Shangai, China	314	1,700	62	31,900	Liu et al., 2010b;
Hong Kong	140	-	ND	19,500	Chung et al., 2007
Harbin, China	301	837	202	3,256	Ma et al., 2009

[a]∑15PAHs

Table 3 - Range of isomer ratios observed in the present study and in other studies around the world. Mean values, when available, are presented between brackets**.**

Local	Matrix	ANT/178	FLA/202	BAA/228	IND/278	Ref.
Lisbon, Portugal	**Urban soils**	**0.10-0.50 (0.19)**	**0.30-0.58 (0.52)**	**0.23-0.58 (0.45)**	**0.26-0.56 (0.47)**	**This study**
Lisbon, Portugal	Urban soils	0.11-0.34 (0.18)	0.50-0.57 (0.53)	0.29-0.53 (0.45)	0.26-0.56 (0.47)	Cachada et al. 2012
Viseu, Portugal	Urban soils	0.01-0.23 (0.14)	0.33-0.63 (0.53)	0.23-0.52 (0.42)	0.27-0.46 (0.40)	
Beijing, China	Urban soils	-	0.5-1.0 (0.59) -	-	- 0.44-0.58	Liu et al. 2010a; Peng et al. 2011
Shangai, China	Urban soils	0.01-0.26	0.11-0.77	0.3-0.6	0.3-0.56	Liu et al. 2010b
Harbin, China	Urban/ Rural soils	0.07-0.19 urb 0.07-0.12 rur	-	0.42-0.49 urb 0.17-0.49 rur	-	Ma et al. 2009
Switzerland	Soils	0.003-0.11 (0.02**)**	0.53-0.72 (0.58)	0.27-0.58 (0.40)	0.36-0.55 (0.49**)**	Brandli et al. 2008
Lisbon, Portugal	Aerosol particles	-	0.48-0.58	0.41-0.52	0.31-0.73	Oliveira et al. 2011
United Kingdom	Ambient air	<0.1	>0.5	0.2-0.4	0.4-0.5	Katsoyiannis et al. 2011
-	-	<0.1 petrogenic >0.1 pyrogenic	<0.4 petrogenic 0-4-0.5 liquid fossil fuel >0.5 coal and biomass	<0.2 petrogenic >0.2 pyrogenic	<0.2 petrogenic 0-2-0.5 liquid fossil fuel >0.5 coal and biomass	Yunker et al. 2002 Brandli et al. 2008

Table 4 - Spearman correlation matrix among PAHs, general parameters and PTEs in Lisbon soils.

	∑PAHs	pH_{Ca}	TC	OC	OM	CEC	Sand	Silt	Clay	Al	As	Ca	Co	Cr	Cu	Hg	Fe	Ni	Pb	Zn
∑PAHs																				
pH_{Ca}	-																			
TC	**0.48**	-																		
OC	**0.40**	-	**0.86**																	
OM	**0.30**	-	**0.83**	**0.91**																
CEC	0.21	-	**0.74**	**0.80**	**0.90**															
Sand	-	-	**-0.40**	**-0.44**	**-0.50**	**-0.60**														
Silt	**0.31**	-	**0.43**	**0.45**	**-0.56**	**0.51**	**-0.89**													
Clay	-	-	**0.27**	**0.31**	**0.43**	**0.58**	**-0.86**	**0.58**												
Al	-	-	**0.35**	**0.36**	**0.49**	**0.70**	**-0.61**	**0.39**	**0.75**											
As	0.35	-	-	-	-	-	-0.21	**0.27**	-	-0.20										
Ca	**0.45**	-	**0.66**	**0.34**	**0.36**	**0.34**	**-0.30**	**0.37**	0.21	**-**	**0.41**									
Co	-	-	**0.44**	**0.42**	**0.52**	**0.73**	**-0.49**	**0.32**	**0.60**	**0.87**	**-0.31**	0.21								
Cr	-	-0.20	**0.46**	**0.41**	**0.53**	**0.71**	**-0.40**	**0.23**	**0.53**	**0.90**	**-0.30**	0.25	**0.87**							
Cu	**0.58**	**-0.31**	**0.48**	**0.45**	**0.44**	**-0.48**	-0.23	0.24	-	**0.44**	-	**0.30**	**0.46**	**0.60**						
Hg	**0.75**	-0.22	**0.42**	**0.31**	0.21	**-**	**-**	0.26	**-**	**-**	**0.31**	**0.38**	**-**	**-**	**0.65**					
Fe	-	-	**0.42**	**0.39**	**0.52**	**0.73**	**-0.54**	**0.35**	**0.66**	**0.96**	-0.25	0.21	**0.91**	**0.93**	**0.54**	-				
Ni	-	-	**0.51**	**0.45**	**0.56**	**0.74**	**-0.48**	**0.30**	**0.54**	**0.86**	**-0.27**	**0.28**	**0.93**	**0.93**	**0.60**	0.21	**0.92**			
Pb	**0.80**	-	**0.48**	**0.38**	**0.28**	0.23	-	**0.33**	-	-	**0.38**	**0.49**	-	-	**0.71**	**0.87**	-	0.25		
Zn	**0.59**	**-0.27**	**0.59**	**0.52**	**0.50**	**0.46**	-0.26	**0.31**	-	**0.35**	-	**0.39**	**0.35**	**0.46**	**0.78**	**0.58**	**0.44**	**0.51**	**0.73**	

Numbers in bold means that correlation is significant at the 0.01 level (2-tailed), for the others correlation is significant at the 0.05 level (2-tailed)

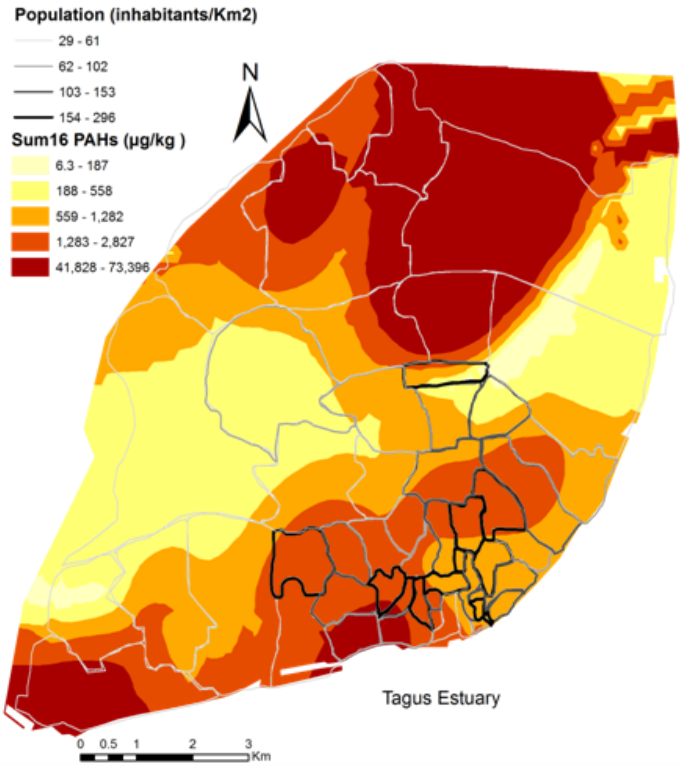


Figure 1 - Contour map interpolated by ordinary kriging of the distribution of the ∑16 PAHs; the class limits corresponds to the minimum, the quartiles (25, 50 and 75), the upper outlier limit, and the maximum value. The map also shows the 53 districts of the city and their population density.

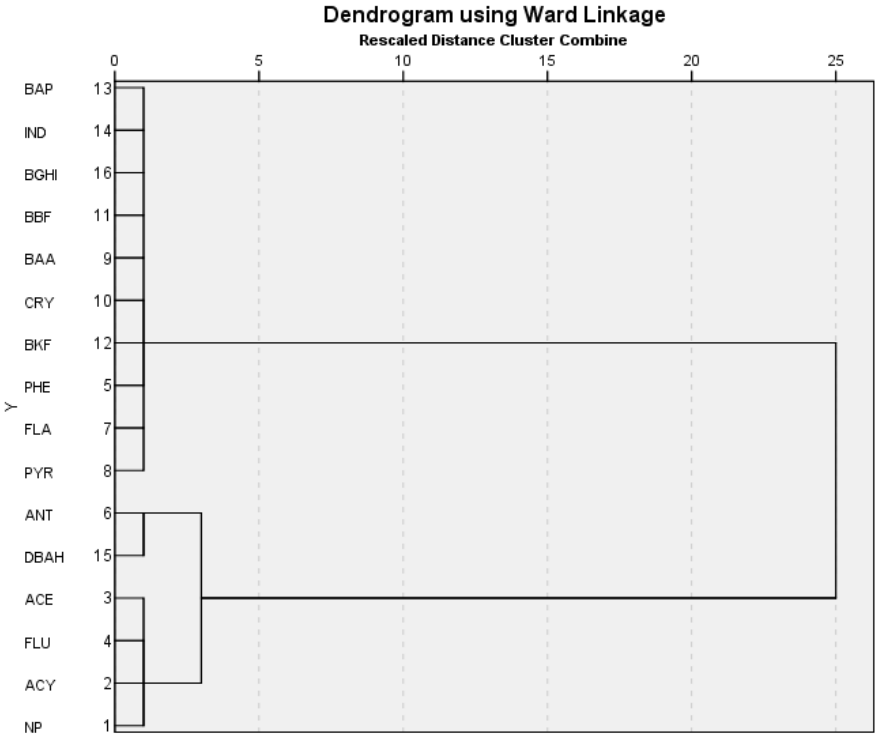


Figure 2 - Dendrogram (Ward method, squared Eucleadian distances, log transformed) of the individual compounds for Lisbon urban soils.

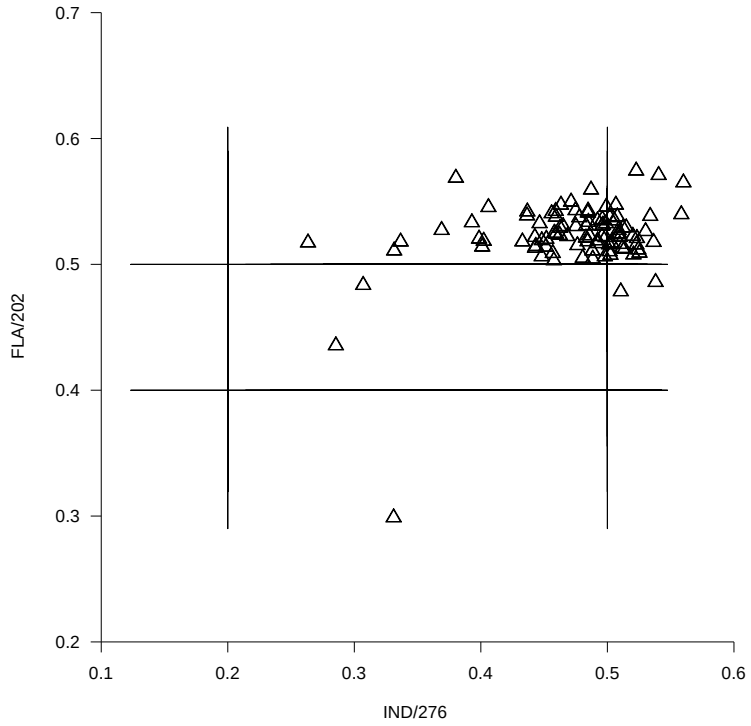


Figure 3 - Isomer ratios of Lisbon soil samples.

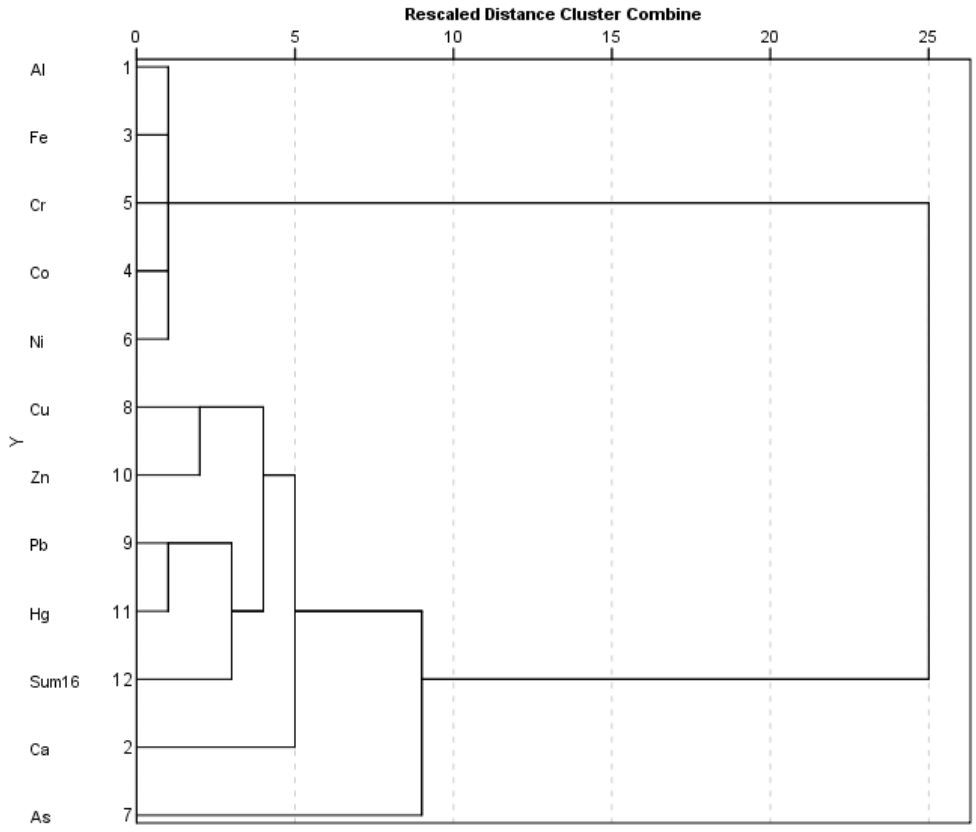


Figure 4 - Dendrogram (Ward method, squared Eucleadian distances, log transformed) of PAHs and PTEs for Lisbon urban soils.

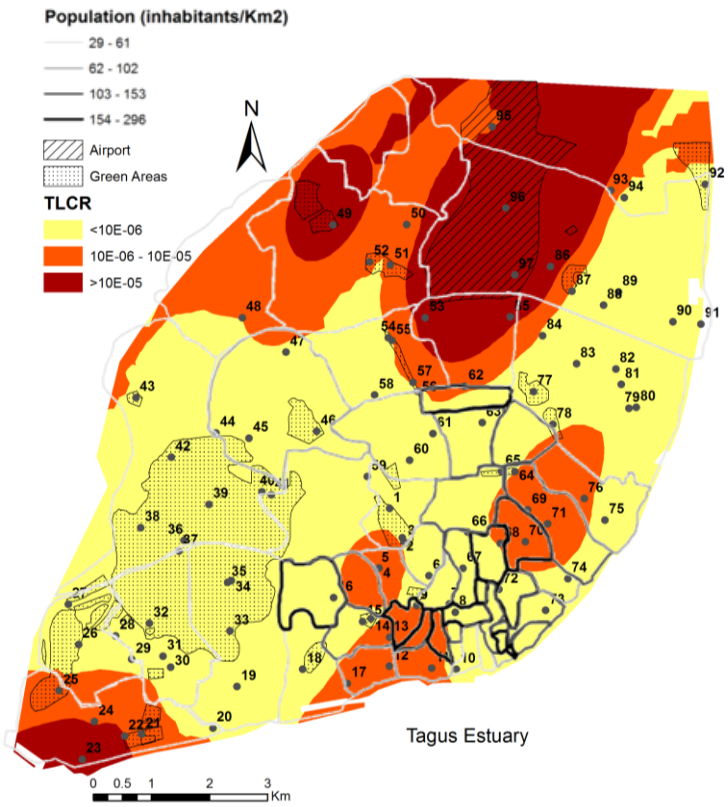


Figure 5 - Contour map interpolated by ordinary kriging for the cancer risks considering a residential land use; the class limits correspond to the risk limits suggested by different guidelines. The map also shows the 53 districts of the city and their population density.

Annex VI

Table 1 – Results of Tenax extractions for individual compounds (µk kg^{-1}) in the 10 samples extracted.

Sample	ACY	ACE	FLU	PHE	ANT	FLA	PYR	BAA	CRY	BBF	BKF	BAP	IND	DBAH	BGHI	SUM
4	bdl	bdl	bdl	4.7	bdl	4.6	4.0	2.1	2.8	3.2	1.3	1.10	1.3	bdl	1.1	26
5	bdl	bdl	bdl	0.36	bdl	0.70	0.66	0.28	1.2	0.73	0.37	bdl	0.41	bdl	0.52	5.2
12	bdl	bdl	bdl	1.4	bdl	3.1	3.1	1.2	3.0	4.6	0.84	1.7	1.5	bdl	1.9	22
18	bdl	bdl	bdl	0.72	bdl	0.84	0.74	0.29	0.64	0.91	0.28	bdl	bdl	bdl	bdl	4.4
22	bdl	bdl	bdl	0.87	bdl	2.0	1.7	0.44	8.1	0.84	0.42	0.33	0.39	bdl	0.49	16
23	bdl	bdl	bdl	3.1	bdl	7.6	6.7	2.4	4.1	5.2	2.2	2.8	3.6	bdl	4.2	42
66	bdl	bdl	bdl	0.65	bdl	0.75	0.68	0.18	4.5	0.41	0.22	0.18	0.16	bdl	0.23	7.9
68	bdl	bdl	bdl	0.79	bdl	0.91	0.69	bdl	1.1	0.63	0.64	0.95	bdl	bdl	bdl	5.7
69	bdl	bdl	bdl	0.43	bdl	0.94	0.83	0.32	1.1	0.55	0.43	0.26	0.08	bdl	0.31	5.2
97	bdl	bdl	bdl	4.1	bdl	25	19	18	55	35	23	15	16	bdl	16	228

bdl=below detection limit

Annex VII

Table 1 - Characterization of soil samples used in the bioaccumulation assay.

Sample	Al %	Ca %	Fe %	Co (mg/kg)	Cr (mg/kg)	Ni (mg/kg)	As (mg/kg)	Cu (mg/kg)	Pb (mg/kg)	Zn (mg/kg)	Hg (mg/kg)
4	0.89	9.27	1.76	10.4	30.0	40.1	4.70	64.6	365.2	123.0	1.81
12	0.90	6.01	2.13	6.8	29.1	26.5	8.05	160.0	427.5	260.5	1.63
17	1.06	9.51	2.01	9.0	42.4	37.9	4.70	133.0	260.0	245.0	1.89
18	0.99	7.98	1.79	8.6	39.0	32.5	3.75	41.5	125.9	128.0	1.15
22	1.90	3.85	3.60	30.8	98.0	97.0	4.70	91.1	137.5	228.0	3.76
23	0.96	5.44	1.79	13.3	44.0	43.8	3.80	34.9	107.7	94.0	0.54
49	0.66	8.56	1.32	5.3	20.7	17.6	2.85	23.3	66.4	71.5	0.25
57	0.98	2.36	1.79	5.3	29.2	21.7	4.73	47.3	119.2	123.0	0.35
66	1.09	4.38	1.84	6.9	30.6	27.7	5.60	56.7	215.0	150.0	1.53

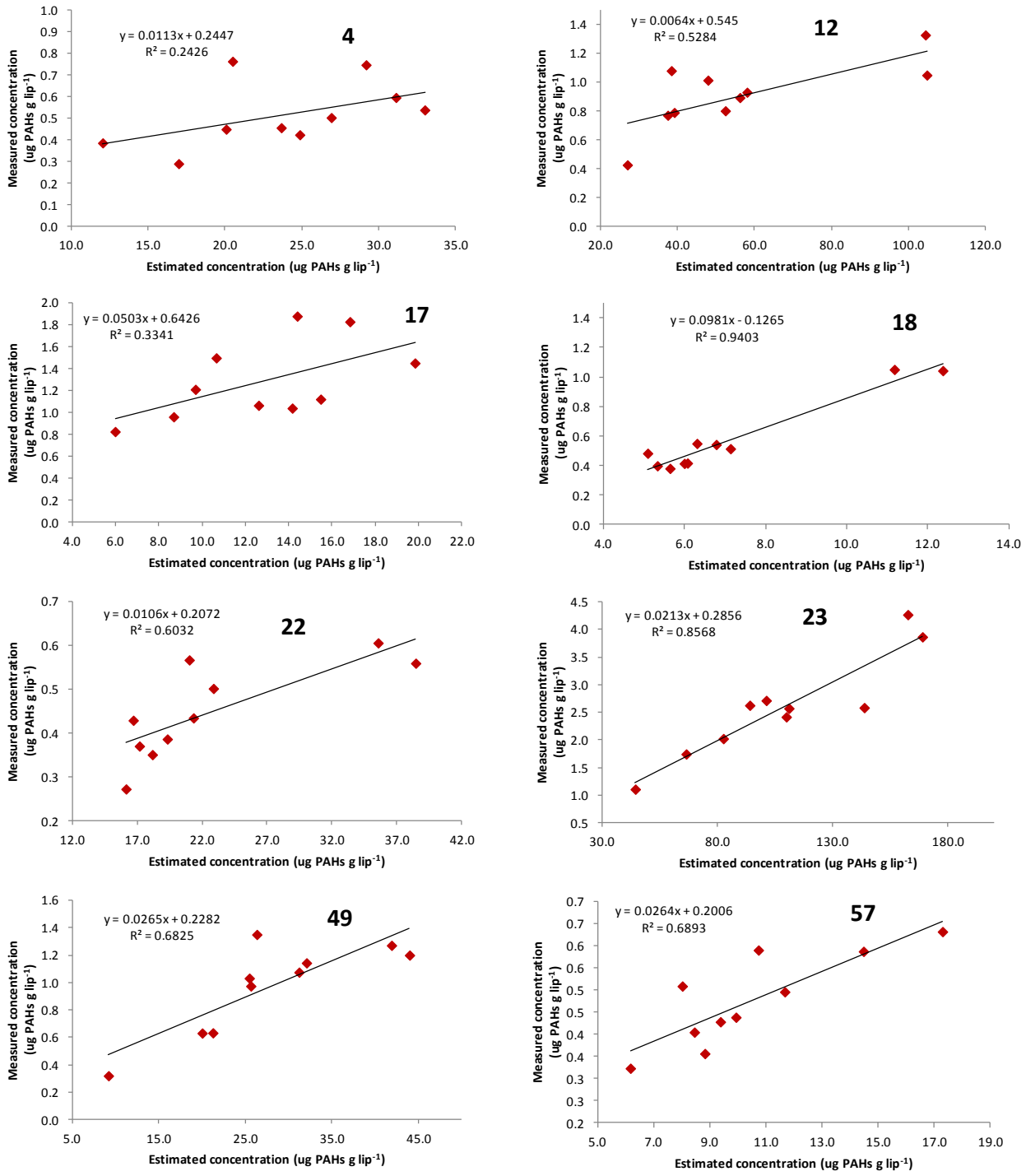


Figure 1 - Relationship between estimated concentration in earthworm using the EqPT and the measured concentrations in each sample.

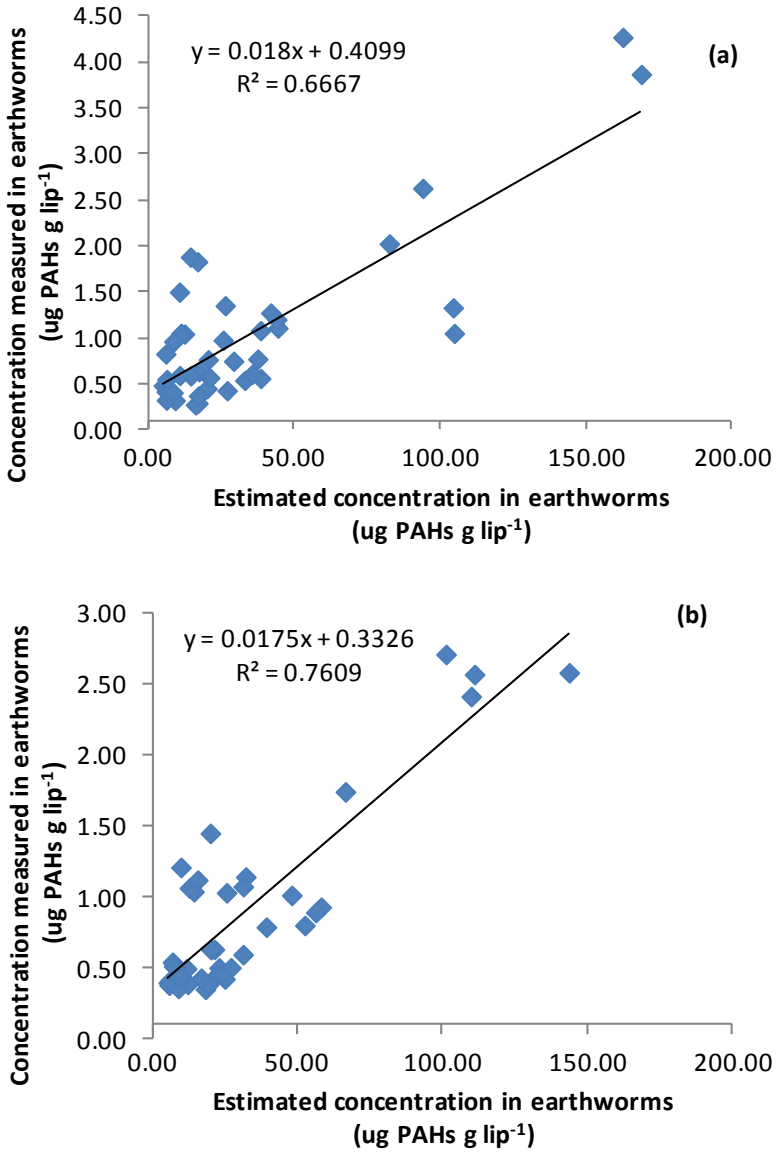


Figure 2 -Relationship between estimated concentration in earthworm using the EqPT and the measured concentrations for 3+4 ring compounds (a) and 5+6 ring compounds (b).

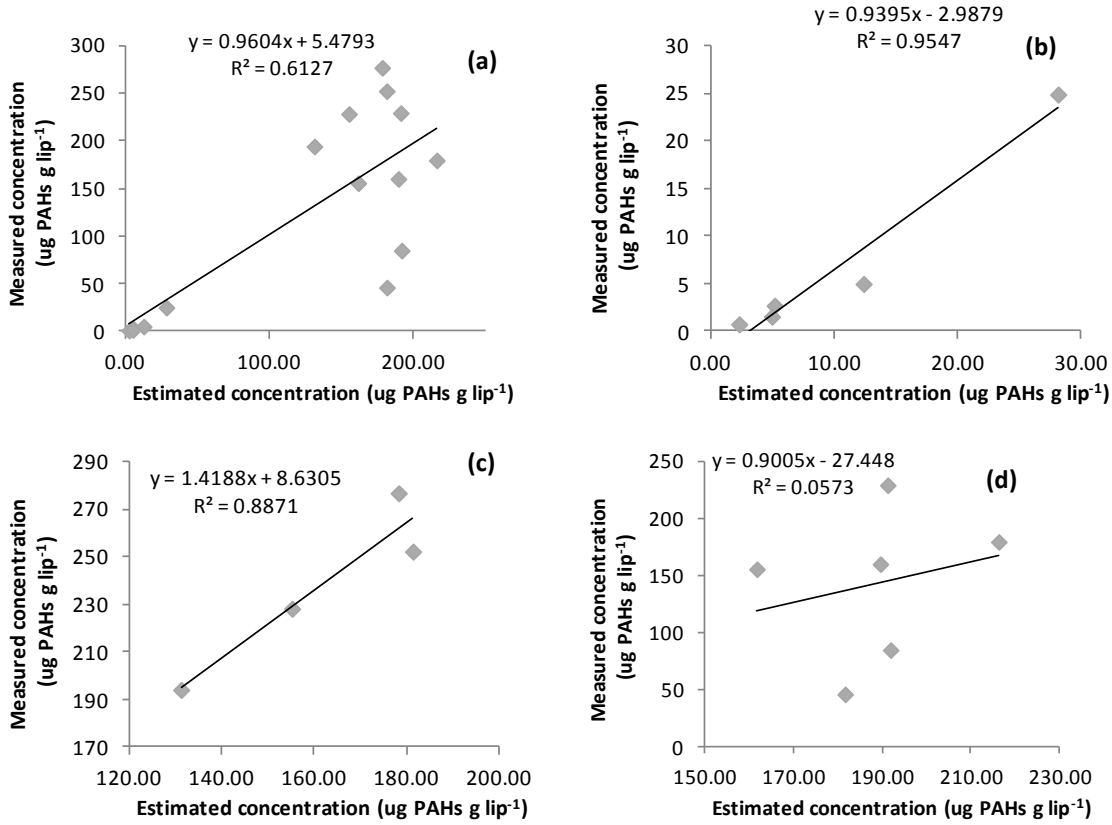


Figure 3 - Relationship between estimated concentration in earthworm using the EqPT and the measured concentrations spiked sample for: the 15 PAHs (a), 3 ring compounds (b); 4ring (c); 5+6 ring (d).

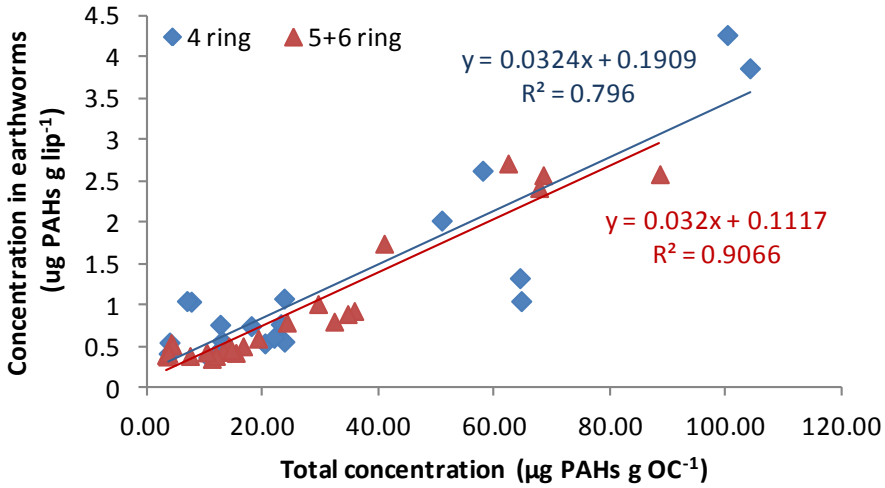


Figure 4 - Relationship between total concentrations in soils and earthworm concentrations for two groups of PAHs in the 5 samples tested for the water soluble fraction vs. earthworm accumulation.